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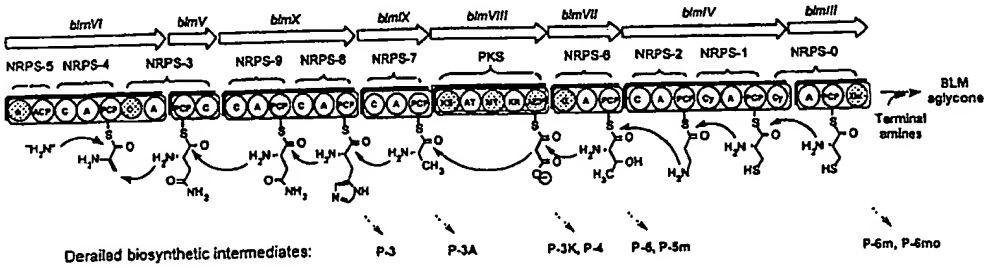

(57) Abstract

This invention provides detailed sequence analysis and characterization of the gene cluster responsible for the synthesis of bleomycin in *Streptomyces verticillus*. The bleomycin gene cluster provides the first hybrid polyketide synthase/nonribosomal peptide synthetase pathway and elucidation of the various modules and enzymatic domains characterizing the pathway provides convenient synthetic routes for bleomycins, bleomycin analogs, and various other polyketides.

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BLEOMYCIN GENE CLUSTER COMPONENTS AND THEIR USES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims benefit under 35 U.S.C. §119 of provisional applications USSN 60/115,435, filed on January 6, 1999, and USSN 60/118,848, filed on February 5, 1999, both of which are herein incorporated by reference in their entirety for all purposes.

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

This work was supported in part by an Institutional Research Grant from the American Cancer Society and the School of Medicien, University of California, Davis, National Institutes of Health Grant Number A140475, and a grant from the Searle Scholars Program of the Chicago Community Trust. The Government of the United States of America may have certain rights in this invention.

FIELD OF THE INVENTION

This invention relates the field of polyketide synthesis and nonribosomal polypeptide synthesis. In particular this invention pertains to the isolation of the bleomycin gene cluster which encodes the first identified hybrid polyketide synthase/nonribosomal peptide synthetase pathway.

BACKGROUND OF THE INVENTION

Polyketides and nonribosomal peptides are two large families of natural products that include many clinically valuable drugs, such as erythromycin and vancomycin (antibacterial), FK506 and cyclosporin (immunosuppresant), and epothilone and bleomycin (BLM) (antitumor). The biosyntheses of polyketides and nonribosomal peptides are catalyzed by polyketide synthases (PKSs) (Hopwood (1997) *Chem. Rev.* 97: 2465; Katz (1997) *Chem. Rev.*, 97: 2557; C. Khosla, (1997) *Chem. Rev.*, 97: 2577; Ikeda and Omura, (1997) *Chem. Rev.*, 97: 2591; Staunton and Wilkinson(1997) *Chem. Rev.*, 97: 2611; Cane *et al.*(1998) *Science* 282: 63) and nonribosomal peptide synthetases (NRPSs) (Cane *et al.*(1998) *Science* 282: 63. Marahiel *et al.* (1997) *Chem. Rev.* 97: 2651; von Döhren *et al.* (1997) *Chem. Rev.* 97: 2675), respectively. Remarkably, PKSs and NRPSs use a very

similar strategy for the assembly of these two distinct classes of natural products by sequential condensation of short carboxylic acids and amino acids, respectively, and utilize the same 4'-phosphopantetheine prosthetic group, via a thioester linkage, to channel the growing polyketide or peptide intermediate during the elongation processes.

Both type I PKSs and NRPSs are multifunctional proteins that are organized into modules. (A module is defined as a set of distinctive domains that encode all the enzyme activities necessary for one cycle of polyketide or peptide chain elongation and associated modifications.) The number and order of modules and the type of domains within a module on each PKS or NRPS protein determine the structural variations of the resulting polyketide and peptide products by dictating the number, order, choice of the carboxylic acid or amino acid to be incorporated, and the modifications associated with a particular cycle of elongation. These features of PKS and NRPS inspired us to search for a hybrid PKS and NRPS system. Since the modular architecture of both PKS (Cane *et al.*(1998) *Science* 282: 63; Katz and Danadio (1993) *Ann. Rev. Microbiol.* 47: 875 (1993); Hutchinson and Fujii (1995) *Ann. Rev. Microbiol.* 49: 201) and NRPS (Cane *et al.*(1998) *Science* 282: 63, Stachelhaus *et al.* (1995) *Science* 269: 69; Stachelhaus *et al.* (198) *Mol. Gen. Genet.* 257: 308; Belshaw *et al.* (1999) *Science* 284, 486) has been exploited successfully in combinatorial biosynthesis of diverse "unnatural" natural products, it is imagined that a hybrid PKS and NRPS system, capable of incorporating both carboxylic acids and amino acids into the final products, could surely lead to even greater chemical structural diversity.

The BLMs, differing structurally at the C-terminal amines of the glycopeptides, are a family of antibiotics produced by *Streptomyces verticillus* (*Sv*). BLMs exhibit strong antitumor activity through a metal-dependent oxidative cleavage of DNA or RNA in the presence of molecular oxygen and are incorporated into current chemotherapy of several malignancies under the trade name of Blenoxane® that contains BLM A2 and BLM B2 as the principal constituents (Sikic *et al.* Eds. (1985) *Bleomycin Chemotherapy,* Academic Press, New York; Natrajan and Hecht (1994) pages 197-242 In: *Molecular Aspects of Anticancer Drug-DNA Interaction Vol. 2*, Neidle and Waring Eds., Macmillan, London). Umezawa, Fujii, Takita, and co-workers extensively studied the biosynthesis of BLM in *Sv* ATCC15003 by feeding isotope-labeled precursors and by isolating various biosynthetic intermediates and shunt metabolites, establishing that the BLMs are in fact natural hybrid metabolites of polyketide and peptide biosynthesis (Takita and Muroka (1990) pages 289-309 In: *Biochemistry of Peptide Antibiotics: Recent Advances in the Biotechnology of β-Lactams and Microbial Peptides*, Kleinkauf and Von Döhren Eds., W. de

Gruyter, New York). On the assumption that BLM biosynthesis follows the paradigm for peptide and polyketide biosynthesis, we predict that the Blm megasynthetase, which catalyzes the assembly of the BLM backbone from nine amino acids and one acetate, should bear the characteristics of both NRPS and PKS, providing an excellent model to study the mechanism by which NRPS and PKS could be integrated into a productive biosynthetic system to synthesize a hybrid peptide and polyketide metabolite (Fig. 1A) (Shen *et al.* (1999) *Bioorg. Chem.* 27: 155).

SUMMARY OF THE INVENTION

This invention pertains to the isolation and elucidation of the bleomycin gene cluster. Nucleic acid sequences encoding all of the open reading frames (ORFs) that encode polypeptides sufficient to direct the biosynthesis of bleomycin are provided. The nucleic acids can be used in their "native" format or recombined in a wide variety of manners to create novel synthetic pathways.

In one embodiment, this invention provides an isolated nucleic acid comprising a nucleic acid selected from the group consisting of a nucleic acid encoding any one of Blm open reading frames (ORFs) 8 through 41, and/or a nucleic acid encoding a polypeptide encoded by any one of Blm open reading frames (ORFs) 8 through 41, and/or a nucleic acid amplified by polymerase chain reaction (PCR) using any one of the primer pairs identified in Table II and the nucleic acid of a bleomycin-producing organism as a template. The nucleic acid may comprise one or multiple (*e.g.* two, more preferably 3 or more) bleomycin open reading frames (*i.e. BLM* ORFs 8 through 41). One preferred nucleic acid comprises a nucleic acid encoding a C domain lacking one or more His residues of the conserved HHxxxDG active site for transpeptidation. In another preferred embodiment the nucleic acid comprises a nucleic acid encoding a protein encoded by a gene selected from the group consisting of blmI, blmII, and blmXI.

In another embodiment this invention provides an isolated nucleic acid encoding a (biosynthetic) module comprising two or more (more preferably three or more, most preferably four or more) catalytic domains of a protein encoded by a nucleic acid of a bleomycin gene cluster wherein said catalytic domains are selected from the group consisting of a condensation (C) domain, an adenylation (A) domain, a peptidyl carrier protein (PCP) domain, a condensation/cyclization domain (Cy), an acyl-carrier protein (ACP)-like domain, an oxidization domain (Ox), a ketoacyl synthase (KS) domain , an acetyl transferase (AT) domain, a ketoreductase (KR) domain, and a methyltransferase (MT) domain. Preferred

nucleic acids comprises a nucleic acid encoding one or more proteins comprising a module selected from the group consisting of NRPS-0, NRPS-1, NRPS-2, NRPS-3, NRPS-4, NRPS-5, NRPS-6, NRPS-7, NRPS-7, NRPS-9, and PKS. Particularly preferred nucleic acids comprise an open reading frame from SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

In still another embodiment, this invention provides an isolated nucleic acid comprising a nucleic acid encoding a protein encoded by a gene from a BLM gene cluster. Preferred nucleic acids encode a protein encoded by a gene selected from the group consisting of blmI, blmII, and blmXI. In another embodiment, preferred nucleic acids encode a protein encoded by a gene selected from the group consisting of blmIII, blmIV, blmV, blmVI, blmVII, blmIX, and blmX. In still yet another embodiment, the nucleic acid comprises a nucleic acid encoding a protein encoded by blmVIII. Particularly preferred nucleic acids comprise a nucleic acid selected from the group consisting of blmI, blmII, and blmXI. Other particularly preferred nucleic acids comprise a nucleic acid selected from the group consisting of blmIII, blmIV, blmV, blmVI, blmVII, blmIX, and blmX, while still other particularly preferred nucleic acids comprise blmVIII.

In still yet another embodiment, this invention provides an isolated nucleic acid comprising a nucleic acid that encodes a protein comprising at least one catalytic domain selected from the group consisting of a condensation (C) domain, an adenylation (A) domain, a peptidyl carrier protein (PCP) domain, a condensation/cyclization domain (Cy), an acyl-carrier protein (ACP)-like domain, an oxidization domain (Ox), a ketoacyl synthase (KS) domain , an acetyl transferase (AT) domain, a ketoreductase (KR) domain, and a methyltransferase (MT) domain, and that hybridizes to a nucleic acid selected from the group consisting of orf8, orf9, orf10, orf11, orf12, orf13, orf14, orf15, orf15, orf16, orf17, orf18, orf19, orf20, orf21, orf22, orf23, orf24, orf25, orf26, orf27, orf28, orf29, orf30, orf31, orf32, orf33, orf34, orf35, orf36, orf37, orf38, orf39, and orf40 under stringent conditions. In certain embodiments this also includes nucleic acids that would stringently hybridizes indicated above, but for, the degeneracy of the nucleic acid code. In other words, if silent mutations could be made in the subject sequence so that it hybridizes to he indicated sequence(s) under stringent conditions, it would be included in certain embodiments. A preferred isolated nucleic acid comprises a nucleic acid encoding a module. A particularly preferred isolated nucleic acid comprises a nucleic acid encoding a BLM gene.

This invention also provides a nucleic acid comprising a nucleic acid selected from the group consisting of consisting of orf8, orf9, orf10, orf11, orf12, orf13, orf14, orf15, orf15, orf16, orf17, orf18, orf19, orf20, orf21, orf22, orf23, orf24, orf25, orf26, orf27, orf28,

orf29, orf30, orf31, orf32, orf33, orf34, orf35, orf36, orf37, orf38, orf39, and orf40, or an allelic variant thereof. Preferred nucleic acids comprise a nucleic acid that is a single nucleotide polymorphism (SNP) of a nucleic acid selected from the group consisting of consisting of orf8, orf9, orf10, orf11, orf12, orf13, orf14, orf15, orf15, orf16, orf17, orf18, orf19, orf20, orf21, orf22, orf23, orf24, orf25, orf26, orf27, orf28, orf29, orf30, orf31, orf32, orf33, orf34, orf35, orf36, orf37, orf38, orf39, and orf40.

This invention also provides an isolated gene cluster comprising open reading frames encoding polypeptides sufficient to direct the assembly of a bleomycin.

In one embodiment this invention provides an isolated multi-functional protein complex comprising both a polyketide synthase (PKS) and a polypeptide synthetase (NRPS) and/or an isolated nucleic acid encoding a multi-functional protein complex comprising both a polyketide synthase (PKS) and a polypeptide synthetase (NRPS).

This invention also provides various *blm* cluster polypeptides or blm cluster-derived polypeptides. Thus, in one embodiment this invention provides an isolated polypeptide comprising a catalytic domain encoded by a nucleic acid of a bleomycin gene cluster wherein said nucleic acid comprises a nucleic acid selected from the group consisting of a nucleic acid encoding any one of Blm open reading frames (ORFs) 8 through 41; and/or a nucleic acid amplified by polymerase chain reaction (PCR) using any one of the primer pairs identified in Table II. Preferred polypeptides comprise an enzymatic domain selected from the group consisting of a condensation (C) domain, an adenylation (A) domain, a peptidyl carrier protein (PCP) domain, a condensation/cyclization domain (Cy), an acyl-carrier protein (ACP)-like domain, an oxidization domain (Ox), a ketoacyl synthase (KS) domain , an acetyl transferase (AT) domain, a ketoreductase (KR) domain, and a methyltransferase (MT) domain. Particularly preferred polypeptides are encoded by the nucleic acids described above and herein.

This invention also provides expression vectors comprising any of the nucleic acids described herein and/or host cells (*e.g.* Streptomyces) transfected and/or transformed with any of these expression vectors. A preferred host cell is transformed with an exogenous nucleic acid comprising a gene cluster encoding polypeptides sufficient to direct the assembly of a bleomycin or bleomycin analog.

This invention also provides methods of use of the *blm* and *blm*-derived nucleic acid(s) and/or polypeptides. One such method is a method of chemically modifying a biological molecule. The method involves contacting a biological molecule that is a substrate for a polypeptide encoded by one or more bleomycin biosynthesis gene cluster

open reading frames with the polypeptide encoded by one or more bleomycin biosynthesis gene cluster open reading frames, whereby the polypeptide chemically modifies the biological molecule. In one particularly preferred embodiment, the biological molecule is an amino acid and said polypeptide is a peptide synthetase. In another preferred embodiment, the polypeptide is a methyl transferase. Other substrates and *blm* encoded polypeptides are illustrated in Table II.

In another embodiment this invention provides a method of coupling a first amino acid to a second amino acid. This method involves contacting the first and second amino acid with a recombinantly expressed bleomycin nonribosomal peptide synthetase (NRPS). A preferred NRPS is selected from the group consisting of NRPS-5, NRPS-4, NRPS-3, NRPS-9, NRPS-8, and NRPS-7. Another preferred NRPS is selected from the group consisting of NRPS-6, NRPS-2, NRPS-1, and NRPS-0. The contacting can be *in* vivo (*e.g.* in a host cell) or *ex vivo.*

In another embodiment this invention provides a methods of coupling a first fatty acid to a second fatty acid, said method comprising contacting the first and second fatty acids with a recombinantly expressed bleomycin polyketide synthase (PKS). Again, the contacting can be *in* vivo (*e.g.* in a host cell) or *ex vivo.*

In still another embodiment, this invention provides a method of producing a bleomycin or bleomycin analog. The method involves providing a cell transformed with an exogenous nucleic acid comprising a bleomycin gene cluster encoding polypeptides sufficient to direct the assembly of said bleomycin or bleomycin analog; culturing the cell under conditions permitting the biosynthesis of bleomycin or bleomycin analog; and isolating said bleomycin or bleomycin analog from said cell.

This invention also provides an isolated nucleic acid comprising a nucleic acid encoding a phosphopantetheinyl transferase said nucleic acid encoding a phosphopantetheinyl transferase being selected from the group consisting of: a nucleic acid encoding the protein encoded by the nucleic acid of SEQ ID NO:3; a nucleic acid amplified by polymerase chain reaction (PCR) using primers that specifically amplify ORF 41 (primers: SEQ ID NO:71 and SEQ ID NO:72) and *Streptomyces* nucleic acid as a template; a nucleic acid encoding a polypeptide having phosphopantetheinyl transferase activity where said nucleic acid specifically hybridizes to the nucleic acid of SEQ ID NO: 3 under stringent conditions. In one embodiment, the nucleic acid comprises the nucleic acid of SEQ ID NO:3.

In another embodiment, this invention provides a polypeptide comprising a phosphopantetheinyl transferase encoded by SEQ ID NO:3 or a polypeptide having phosphopantetheinyl transferase activity and the sequence encoded by the nucleic acid of SEQ ID NO: 3 or conservative substitutions of that polypeptide.

Also provided are vectors comprising a nucleic acid encoding a phosphopantetheinyl transferase (*e.g.*, as described above) and cells transfected with the vector.

This invention also provides a method of converting an apo carrier protein to a holo carrier protein, said method comprising reacting said apo-carrier protein with a recombinant phosphopantetheinyl transferase encoded by SEQ ID NO:3 and coenzyme A thereby producing a holo-carrier protein.

In certain embodiments, this invention specifically excludes one or more of open reading frames 1 through 41. In particularly preferred embodiments, this invention excludes open reading frames 1 through 7 (Orf 1- Orf 7).

DEFINITIONS

The "polyketide synthases" (PKSs) refers are multifunctional enzymes, related to fatty acid synthases (FASs). PKSs catalyze the biosynthesis of polyketides through repeated (decarboxylative) Claisen condensations between acylthioesters, usually acetyl, propionyl, malonyl or methylmalonyl. Following each condensation, they typically introduce structural variability into the product by catalyzing all, part, or none of a reductive cycle comprising a ketoreduction, dehydration, and enoylreduction on the β-keto group of the growing polyketide chain. PKSs incorporate enormous structural diversity into their products, in addition to varying the condensation cycle, by controlling the overall chain length, choice of primer and extender units and, particularly in the case of aromatic polyketides, regiospecific cyclizations of the nascent polyketide chain. After the carbon chain has grown to a length characteristic of each specific product, it is typically released from the synthase by thiolysis or acyltransfer. Thus, PKSs consist of families of enzymes which work together to produce a given polyketide. Two general classes of PKSs exist. One class, known as Type I PKSs, is represented by the PKSs for macrolides such as erythromycin. These "complex" or "modular" PKSs include assemblies of several large multifunctional proteins carrying, between them, a set of separate active sites for each step of carbon chain assembly and modification (Cortes *et al.* (1990) *Nature* 348: 176; Donadio *et al.* (1991) *Science* 252: 675; MacNeil *et al.* (1992) *Gene* 115: 119). Structural diversity

occurs in this class from variations in the number and type of active sites in the PKSs. This class of PKSs displays a one-to-one correlation between the number and clustering of active sites in the primary sequence of the PKS and the structure of the polyketide backbone. The second class of PKSs, called Type II PKSs, is represented by the synthases for aromatic compounds. Type II PKSs typically have a single set of iteratively used active sites (Bibb *et al.* (1989) *EMBO J.* 8: 2727; Sherman *et al.* (1989(*EMBO J.* 8: 2717; Fernandez-Moreno, *et al.* (1992) *J. Biol. Chem.* 267:19278).

A "nonribosomal peptide synthase" (NRPS) refers to an enzymatic complex of eucaryotic or procaryotic origin, that is responsible for the synthesis of peptides by a nonribosomal mechanism, often known as thiotemplate synthesis (Kleinkauf and von Doehren (1987) *Ann. Rev. Microbiol.*, 41: 259-289). Such peptides, which can be up to 20 or more amino acids in length, can have a linear, cyclic (cyclosporine, tyrocidine, mycobacilline, surfactin and others) or branched cyclic structure (polymyxin, bacitracin and others) and often contain amino acids not present in proteins or modified amino acids through methylation or epimerization.

A "module" refers to a set of distinctive polypeptide domains that encode all the enzyme activities necessary for one cycle of polyketide or peptide chain elongation and associated modifications.

The terms "isolated" "purified" or "biologically pure" refer to material which is substantially or essentially free from components which normally accompany it as found in its native state. With respect to nucleic acids and/or polypeptides the term can refer to nucleic acids or polypeptides that are no longer flanked by the sequences typically flanking them in nature.

The terms "polypeptide", "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers. The term also includes variants on the traditional peptide linkage joining the amino acids making up the polypeptide.

The terms "nucleic acid" or "oligonucleotide" or grammatical equivalents herein refer to at least two nucleotides covalently linked together. A nucleic acid of the present invention is preferably single-stranded or double stranded and will generally contain phosphodiester bonds, although in some cases, as outlined below, nucleic acid analogs are included that may have alternate backbones, comprising, for example, phosphoramide

(Beaucage *et al.* (1993) *Tetrahedron* 49(10):1925) and references therein; Letsinger (1970) *J. Org. Chem.* 35:3800; Sprinzl et al. (1977) *Eur. J. Biochem.* 81: 579; Letsinger *et al.* (1986) *Nucl. Acids Res.* 14: 3487; Sawai *et al.* (1984) *Chem. Lett.* 805, Letsinger *et al.* (1988) *J. Am. Chem. Soc.* 110: 4470; and Pauwels *et al.* (1986) *Chemica Scripta* 26: 1419), phosphorothioate (Mag et al. (1991) *Nucleic Acids Res.* 19:1437; and U.S. Patent No. 5,644,048), phosphorodithioate (Briu *et al.* (1989) *J. Am. Chem. Soc.* 111 :2321, O-methylphophoroamidite linkages (*see* Eckstein, *Oligonucleotides and Analogues: A Practical Approach*, Oxford University Press), and peptide nucleic acid backbones and linkages (*see* Egholm (1992) *J. Am. Chem. Soc.* 114:1895; Meier *et al.* (1992) *Chem. Int. Ed. Engl.* 31: 1008; Nielsen (1993) *Nature*, 365: 566; Carlsson *et al.* (1996) *Nature* 380: 207). Other analog nucleic acids include those with positive backbones (Denpcy *et al.* (1995) *Proc. Natl. Acad. Sci. USA* 92: 6097; non-ionic backbones (U.S. Patent Nos. 5,386,023, 5,637,684, 5,602,240, 5,216,141 and 4,469,863; Angew. (1991) *Chem. Intl. Ed. English* 30: 423; Letsinger et al. (1988) *J. Am. Chem. Soc.* 110:4470; Letsinger *et al.* (1994) *Nucleoside & Nucleotide* 13:1597; Chapters 2 and 3, ASC Symposium Series 580, "Carbohydrate Modifications in Antisense Research", Ed. Y.S. Sanghui and P. Dan Cook; Mesmaeker *et al.* (1994), *Bioorganic & Medicinal Chem. Lett.* 4: 395; Jeffs *et al.* (1994) *J. Biomolecular NMR* 34:17; *Tetrahedron Lett.* 37:743 (1996)) and non-ribose backbones, including those described in U.S. Patent Nos. 5,235,033 and 5,034,506, and Chapters 6 and 7, ASC Symposium Series 580, *Carbohydrate Modifications in Antisense Research*, Ed. Y.S. Sanghui and P. Dan Cook. Nucleic acids containing one or more carbocyclic sugars are also included within the definition of nucleic acids (*see* Jenkins *et al.* (1995), *Chem. Soc. Rev.* pp169-176). Several nucleic acid analogs are described in Rawls, C & E News June 2, 1997 page 35. These modifications of the ribose-phosphate backbone may be done to facilitate the addition of additional moieties such as labels, or to increase the stability and half-life of such molecules in physiological environments.

The term "heterologous" as it relates to nucleic acid sequences such as coding sequences and control sequences, denotes sequences that are not normally associated with a region of a recombinant construct, and/or are not normally associated with a particular cell. Thus, a "heterologous" region of a nucleic acid construct is an identifiable segment of nucleic acid within or attached to another nucleic acid molecule that is not found in association with the other molecule in nature. For example, a heterologous region of a construct could include a coding sequence flanked by sequences not found in association with the coding sequence in nature. Another example of a heterologous coding sequence is a

construct where the coding sequence itself is not found in nature (e.g., synthetic sequences having codons different from the native gene). Similarly, a host cell transformed with a construct which is not normally present in the host cell would be considered heterologous for purposes of this invention.

A "coding sequence" or a sequence which "encodes" a particular polypeptide (*e.g.* a PKS, an NRPS, *etc.*), is a nucleic acid sequence which is ultimately transcribed and/or translated into that polypeptide *in vitro* and/or *in vivo* when placed under the control of appropriate regulatory sequences. In certain embodiments, the boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. A coding sequence can include, but is not limited to, cDNA from procaryotic or eucaryotic mRNA, genomic DNA sequences from procaryotic or eucaryotic DNA, and even synthetic DNA sequences. In preferred embodiments, a transcription termination sequence will usually be located 3' to the coding sequence.

Expression "control sequences" refers collectively to promoter sequences, ribosome binding sites, polyadenylation signals, transcription termination sequences, upstream regulatory domains, enhancers, and the like, which collectively provide for the transcription and translation of a coding sequence in a host cell. Not all of these control sequences need always be present in a recombinant vector so long as the desired gene is capable of being transcribed and translated.

"Recombination" refers to the reassortment of sections of DNA or RNA sequences between two DNA or RNA molecules. "Homologous recombination" occurs between two DNA molecules which hybridize by virtue of homologous or complementary nucleotide sequences present in each DNA molecule.

The terms "stringent conditions" or "hybridization under stringent conditions" refers to conditions under which a probe will hybridize preferentially to its target subsequence, and to a lesser extent to, or not at all to, other sequences. "Stringent hybridization" and "stringent hybridization wash conditions" in the context of nucleic acid hybridization experiments such as Southern and northern hybridizations are sequence dependent, and are different under different environmental parameters. An extensive guide to the hybridization of nucleic acids is found in Tijssen (1993) *Laboratory Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Acid Probes part I chapter 2 Overview of principles of hybridization and the strategy of nucleic acid probe assays*, Elsevier, New York. Generally, highly stringent hybridization and wash conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence

at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Very stringent conditions are selected to be equal to the T_m for a particular probe.

An example of stringent hybridization conditions for hybridization of complementary nucleic acids which have more than 100 complementary residues on a filter in a Southern or northern blot is 50% formamide with 1 mg of heparin at 42°C, with the hybridization being carried out overnight. An example of highly stringent wash conditions is 0.15 M NaCl at 72°C for about 15 minutes. An example of stringent wash conditions is a 0.2x SSC wash at 65°C for 15 minutes (*see*, Sambrook *et al.* (1989) *Molecular Cloning - A Laboratory Manual (2nd ed.)* Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor Press, NY, for a description of SSC buffer). Often, a high stringency wash is preceded by a low stringency wash to remove background probe signal. An example medium stringency wash for a duplex of, *e.g.*, more than 100 nucleotides, is 1x SSC at 45°C for 15 minutes. An example low stringency wash for a duplex of, *e.g.*, more than 100 nucleotides, is 4-6x SSC at 40°C for 15 minutes. In general, a signal to noise ratio of 2x (or higher) than that observed for an unrelated probe in the particular hybridization assay indicates detection of a specific hybridization. Nucleic acids which do not hybridize to each other under stringent conditions are still substantially identical if the polypeptides which they encode are substantially identical. This occurs, *e.g.*, when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code.

A "library" or "combinatorial library" of polyketides and/or polypeptides is intended to mean a collection of polyketides and/or polypeptides (or other molecules) catalytically produced by a PKS and/or NRPS and/or hybrid PKS/NRPS (or other possible combination of synthetic elements) gene cluster. The library can be produced by a gene cluster that contains any combination of native, homolog or mutant genes from aromatic, modular or fungal PKSs and/or NRPSs. The combination of genes can be derived from a single PKS and/or NRPS gene cluster, *e.g.*, *act, fren, gra, tcm, whiE, gris, ery*, or the like, and may optionally include genes encoding tailoring enzymes which are capable of catalyzing the further modification of a polypeptide, polyketide, or other molecule. Alternatively, the combination of genes can be rationally or stochastically derived from an assortment of NRPS and/or PKS gene clusters. The library of polyketides and/or polypeptides and/or other molecules thus produced can be tested or screened for biological, pharmacological or other activity.

By "random assortment" is intended any combination and/or order of genes, homologs or mutants which encode for the various PKS and/or NRPS enzymes, modules, active sites or portions thereof derived from aromatic, modular or fungal PKS and/or NRPS gene clusters.

By "genetically engineered host cell" is meant a host cell where the native PKS and/or NRPS gene cluster has been altered or deleted using recombinant DNA techniques or a host cell into which a heterologous PKS and/or NRPS and/or hybrid PKS/NRPS gene cluster has been inserted. Thus, the term would not encompass mutational events occurring in nature. A "host cell" is a cell derived from a procaryotic microorganism or a eucaryotic cell line cultured as a unicellular entity, which can be, or has been, used as a recipient for recombinant vectors bearing the PKS, NRPS, and/or hybrid gene clusters of the invention. The term includes the progeny of the original cell which has been transfected. It is understood that the progeny of a single parental cell may not necessarily be completely identical in morphology or in genomic or total DNA complement to the original parent, due to accidental or deliberate mutation. Progeny of the parental cell which are sufficiently similar to the parent to be characterized by the relevant property, such as the presence of a nucleotide sequence encoding a desired PKS, are included in the definition, and are covered by the above terms.

Expression vectors are defined herein as nucleic acid sequences that are direct the transcription of cloned copies of genes/cDNAs and/or the translation of their mRNAs in an appropriate host. Such vectors can be used to express genes or cDNAs in a variety of hosts such as bacteria, bluegreen algae, plant cells, insect cells and animal cells. Expression vectors include, but are not limited to, cloning vectors, modified cloning vectors, specifically designed plasmids or viruses. Specifically designed vectors allow the shuttling of DNA between hosts, such as bacteria-yeast or bacteria-animal cells. An appropriately constructed expression vector preferably contains: an origin of replication for autonomous replication in a host cell, a selectable marker, optionally one or more restriction enzyme sites, optionally one or more constitutive or inducible promoters. In preferred embodiments, an expression vector is a replicable DNA construct in which a DNA sequence encoding a one or more PKS and/or NRPS domains and/or modules is operably linked to suitable control sequences capable of effecting the expression of the products of these synthase and/or synthetases in a suitable host. Control sequences include a transcriptional promoter, an optional operator sequence to control transcription and sequences which control the termination of transcription and translation, and so forth.

A "bleomycin open reading frame", or "bleomycin ORF", or "*BLM* Orf" refers to a nucleic acid open reading frame that encodes a polypeptide or polypeptide domain that has an enzymatic activity used in the biosynthesis of a bleomycin.

A "PKS/NRPS/PKS" system refers to a synthetic system comprising an NRPS flanked by two PKSs. A "NRPS/PKS/NRPS" system refers to a synthetic system comprising a PKS flanked by two NRPSs. A "hybrid PKS/NRPS system" or a "hybrid NRPS/PKS system" refers to a hybrid synthetic system comprising at least one PKS and one NRPS module. The system can comprise multiple modules and the order can vary.

A "biological molecule that is a substrate for a polypeptide encoded by a bleomycin biosynthesis gene" refers to a molecule that is chemically modified by one or more polypeptides enccoded by open reading frame(s) of the *blm* gene cluster. The "substrate" may be a native molecule that typically participates in the biosynthesis of a bleomycin, or can be any other molecule that can be similarly acted upon by the polypeptide.

A "polymorphism" is a variation in the DNA sequence of some members of a species. A polymorphism is thus said to be "allelic," in that, due to the existence of the polymorphism, some members of a species may have the unmutated sequence (*i.e.* the original "allele") whereas other members may have a mutated sequence (*i.e.* the variant or mutant "allele"). In the simplest case, only one mutated sequence may exist, and the polymorphism is said to be diallelic. In the case of diallelic diploid organisms, three genotypes are possible. They can be homozygous for one allele, homozygous for the other allele or heterozygous. In the case of diallelic haploid organisms, they can have one allele or the other, thus only two genotypes are possible. The occurrence of alternative mutations can give rise to trialleleic, *etc.* polymorphisms. An allele may be referred to by the nucleotide(s) that comprise the mutation.

"Single nucleotide polymorphism" or "SNPs are defined by their characteristic attributes. A central attribute of such a polymorphism is that it contains a polymorphic site, "X," most preferably occupied by a single nucleotide, which is the site of the polymorphism's variation (Goelet and Knapp U.S. patent application Ser. No. 08/145,145). Methods of identifying SNPs are well known to those of skill in the art (*see, e.g.*, U.S. Patent 5,952,174).

The following abbreviations are used herein:: A, adenylation; ACP, acyl carrier protein; AT, acyltransferase; BLM, bleomycin; C, condensation; Cy, condensation/cyclization; KR, ketoreductase; KS, ketoacyl synthase; MT, methyltransferase; NRPS, nonribosomal peptide synthetase; orf, open reading frame; Ox, oxidation; PCP,

peptidyl carrier protein; PCR, polymerase chain reaction; PKS, polyketide synthase; *Sv*, *Streptomyces verticillus*, ArCP, aryl carrier protein, bp, base pair, CoA, co-enzyme A, DTT, dithiothreitol; FAS, fatty acid synthase; kb, kilobase; PPTase, 4'-phosphopantetheinyl transferase; TCA, trichloroacetic acid; and DEBS, 6-deoxyerythronolide B synthase..

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B illustrate the biosynthetic pathway for bleomycin in *Sv* (ATCC 15003). Figure 1A illustrates a biosynthetic pathway for BLM in *Sv* ATCC15003–intermediates except those in brackets were identified. Figure 1B shows a linear model for the Blm megasynthetase-templated assembly of the BLM peptide/polyketide/peptide aglycone from nine amino acids and one acetate–shaded circles represent atypical domains carrying out the proposed novel chemistry, and arrows with broken line indicate where biosynthetic intermediates were derailed. Three-letter amino acid designations were used. [HO], hydroxylation; [H], reduction.

Figure 2 provides a restriction map and gene organization of the *blm* gene cluster from *Sv* ATCC15003 (B, *Bam*HI). Proposed functions for individual open reading frames are summarized in Tables I and II. Modules for individual NRPS and PKS were given along with their proposed substrates in parentheses.

Figures 3A, 3B, 3C, and 3D illustrate the determination of substrate specificity for NRPS-1 and NRPS-6. Figure 3A shows a comparison of the A3 to A6 region of A domains to 84 NRPS modules available at GenBank that activate various amino acids. Figure 3B shows a comparison of amino acid residues that putatively line the substrate binding pockets for A domains (single-letter amino acid designations were used). The number following the protein name indicates the order of a particular A domain in the multimodular NRPS protein. The protein accession numbers are P48663 (HMWP2), P19828 (AngR), AAC06346 (BacA-2), CAB03756 (MbtB), 3510629 (SyrE-7), 3114612 (AcmB-1), CAA67248 (SnbC-1), and 3560507 (FxbC-2). Dhb stands for 2,3-dehydroaminobutyric acid. It is not known if Dhb is the direct substrate for SyrE-7 or resulted from dehydration of an SyrE-7 activated Thr (Guenzi *et al.* (1998) *J. Biol. Chem.* 273: 32857-32863). Figure 3C illustrates purified proteins after overexpression in *E. coli* as analyzed by electrophoresis on a 10% SDS-polyacrylamide gel (the calculated molecular weights for NRPS-1A and NRPS-6A are 64,212 and 61,899, respectively). Figure 3D illustrates substrate specificities as determined by the ATP-PPi exchange reaction with the amino acids of BLM as substrates

(100% relative activity corresponds to 103,000 cpm for NRPS-1A and 256,000 cpm for NRPS-6A).

Figure 4 illustrates a three-module NRPS/PKS/NRPS model for channeling the growing intermediate between NRPS and PKS modules and between PKS and NRPS modules. The KS, ACP, and C domains are shaded to emphasize their unique activities that are responsible for elongating a growing peptide with a short carboxylic acid and a growing polyketide with an amino acid in hybrid peptide/polyketide/peptide biosynthesis.

Figure 5 illustrates the use of *blmVIII* methyltransferase domain to introduce branched methyl groups in a polyketide synthesis. PCK12 has been described by Kao *et al.* (1995) *J. Am. Chem. Soc.*, 7: 9105-9106. DE-1, DE-2 and DE-3 rae three representative products demonstrating the strategy and utility of *blmVIII* in introducing a CH_3 group in polyketide biosynthesis.

Figure 6 illustrates the use of the *blm* NRPS and PKS enzymes to synthesize a variety of hybrid polyketide/peptide molecules including, but not limited to, a family of oxazolines/oxazoles, and thiazoline/thiazoles.

Figure 7 illustrates the use of elements of the *blm* gene cluster to synthesize various sugars.

Figure 8Ashows a restriction map of the *blm* gene cluster from *Sv* ATCC15003 (B, *Bam*HI). 8B shows the relative position of the *blmI*, *blmII*, and *blmXI* genes to the two *blmAB* resistance genes (blm^R, Blm resistance). Individual open reading frames are represented by open arrows. Figure 8C shows the nucleotide sequence of the *blmI* gene. The potential ribosome-binding site (RBS) and the conserved motif for 4'-phosphopantetheinylation are underlined. The sequence has been deposited into GenBank under accession no. _____.

Figure 9 shows an amino acid sequence comparison of BlmI with PCP domains of known type I NRPSs (Grs-2 [P14688], 36% identity, 58% similarity; Srfa-3 [Q08787], 40% identity, 64% similarity; Vir-s [Y11547], 36% identity, 60% similarity; Saf-b [U24657], 40% identity, 54% similarity). Given in brackets are nucleotide sequence accession numbers. The shaded letters indicate similar amino acids. Consensus residues are amino acids that are similar in more than three sequences. The signature motif for 4'-phosphopantetheinylation is underlined.

Figures 10A and 10B shows the HPLC analysis of BlmI purified from *E. coli* OG7001(pBS2) (Fig. 10A), and *E. coli* OG7001(pBS2/pDPT-Gsp) (Fig. 10B).

Figure 11 shows the enzyme architecture of type I and type II PKS and NRPS. A, adenylation domain; ACP, acyl carrier protein or ACP domain; AT, acyl transferase; C, condensation protein or C domain; KS, β-ketoacyl synthase domain; KSα, β-ketoacyl synthase α subunit; KSβ, β-ketoacyl synthase β subunit; PCP, peptidyl carrier protein or PCP domain.

Figure 12 illustrates the reaction catalyzed by phosphopantetheinyl transferases (PPTases).

Figure 13 shows a restriction map and gene organization of the *pptA* locus from *Sv* ATCC15003

DETAILED DESCRIPTION

Polyketides and polypeptides can be assembled in a remarkably similar manner by repetitive addition of an extending unit to a growing chain by polyketide synthases (PKS) and nonribosomal peptide synthetase (NRPS) respectively. In the case of polyketides, the extending unit is typically a fatty acid (activated as an acyl CoA thioester) while the extending unit for polypeptides is typically an amino acid (activated as an aminonacyl adenylate). Both the PKS and NRPS systems have evolved a modular organization to define the number, sequence, and specificity of the incorporation of the extending unit and utilized the 4'-phosphopanththeine prosthetic group to channel the growing intermediate during the elongation process.

This invention pertains to the discovery that a PKS-bound growing polyketide intermediate could be further elongated by an NRPS module, or conversely, a NRPS-bound growing polypeptide intermediate can be further elongated by a PKS module. This discovery permits the exploitation of NPRS, PKS, and hybrid NRPS/PKS systems to provide a number of novel hybrid peptide/polyketide metabolites from amino acids and short fatty acids.

It was also a discovery of this invention that this hybrid NRPS/PKS/NRPS system is exemplified by the bleomycin (Blm) biosynthesis pathway in *Streptomyces verticillus* (*Sv.*) (ATCC 15003). The bleomycins are a family of glycopeptide-derived antibiotics originally isolated by Umezawa in 1996 from the fermentation broth of *S. verticillus*. Bleomycins (BLMs) exhibit strong anti-tumor activity are currently used in the treatment of lymphoma, particularly Hodgkin's disease, testicular tumors, squamous cell carcinomas of skin, head, cervix, penis, rectum, and for intracavitary therapy of malignant effusions in ovarian and breast cancer. The commercial product, Blenoxane®, contains

BLM A2 and B2 as the principle constituents. Almost uniquely among anticancer drugs, BLM does not cause myelosuppression, promoting its wide application in combination chemotherapy.

In one aspect, this invention provides a cloned and characterized BLM gene cluster consisting of characteristic NRPS and PKS genes from the Blm producer *Streptoveticillum sp.* (ATCC 15003). The cloned and isolated Blm gene cluster provides a method of recombinantly expressing bleomycin and/or bleomycin analogues. Thus, in one embodiment, this invention provides for nucleic acids encoding bleomycin synthetic machinery or subunits thereof, for cells recombinantly modified to express a bleomycin and/or bleomycin analogue, and for a bleomycin or bleomycinh analogue recombinantly expressed in such cells.

Like other polyketide synthase or nonribosomal peptide synthetases, the bleomycin synthetic pathway is organized into modules, each module catalyzing the addition and/or modification of one subunit (*e.g.* fatty acid or amino acid). Each module is organized into a number of domains each domain having a characteristic activity (*e.g.* activation, condensation, condensation/cyclization, *etc.*). The catalytic domains within a module and the modules themselves are often arranged collinearly and the order of biosynthetic modules from NH_2- to COOH-terminus on each PKS and NRPS polypeptide and the number and type of catalytic domains within each determine the order of structural and functional elements in the resulting product. The size and complexity of the ultimately formed product are controlled by the number of repeated acyl chain extension steps that are, in turn, a function of the number and placement of carrier protein domains in these multimodular enzymes. The number composition and order of such domains can be altered either to introduce modifications, *e.g.* into the bleomycin to produce bleomycin analogues, or to produce different or completely new molecules. Such "recombination" is not restricted solely to recombination among the bleomycin catalytic domains and/or modules, but can also involve recombination between beomycin modules and/or subunits and other PKS and/or NRPS modules and/or subunit. Moreover the discovery that synthetic pathways can incorporate both PKS and NRPS modules and/or catalytic domains makes available hybrid PKS/NRPS syntheses.

Thus, in one embodiment this invention contemplates the use of *blm* gene cluster modules and/or catalytic domains to make various peptide and/or polyketide, and/or hybrid polypeptide/polyketide metabolites (including, but not limited to bleomycin

intermediates or shunt metabolites), in combinatorial biosynthesis with other polyketide synthases and/or other nonribosomal peptide synthetases.

The *blm* gene cluster contains several glycosylases which can be used alone or in context with other PKS and/or NRPS modules or catalytic domains to make various metabolites with sugars associated with bleomycins (bleomycin sugars).

In addition, the *blm* gene cluster includes a novel methyltransferase domain that can be used to make polyketide metabolites with methyl branch(s).

The *blm* gene cluster also is characterized by the unusual Cy domains as well as the unprecedented Ox domain (*see, e.g.* BlmIV and BlmIII NRPSs), providing an efficient biosynthesis for a bithiazole structure. The *blm* gene cluster, *blm* modules, or *blm* catalytic domains can be used either individually or collectively (alone or in combinations with other nonribosomal peptide synthetases or polyketide synthases) to make thiazolidine, thiazoline and thiazole, bi-thiazolidine, bithiazoline, and bithiazole-containing microbioal metabolites.

Other uses include, but are not limited to the usage of the *blm* gene cluster/modules/catalytic units (either individually or collectively) or the Blm model to make heterocyclic ring-containing microbioal metabolites, such as five member S- and N-containing compounds of the thiazolidine, thiazoline and thiazole family or the O- and N-containing compounds of the oxazolidine, oxazoline, and oxazole family or to make sugars, such L-sugars (with the BlmG epimerase), sugars modified by carbamoyl group (with BlmD), and disaccharides.

This invention also includes the discovery of a novel discrete PCP protein (encoded by the *BlmI* gene). Apo-BlmI can be efficiently modified into holo-BlmI either *in vivo* or *in vitro* by PCP-specific 4'-phosphopantetheine transferases (PPTases) such as Gsp and Sfp. Unlike the PCP domains in type I NRPSs, blmI lacks its cognate A domain and can be aminoacylated by Val-A, an A domain from a completely unrelated type I NRPS. BlmI, therefore, represents the first characterized bype II PCP, providing the genetic and biochemical evidence to support the existence of a bype II NRPS. The latter system is useful, in a manner analogous to the type I NRPS, *i.e.*, modular NRPS, in the combinatorial manipulation of NRPS proteins to generate novel peptides. This invention also includes the discovery and characterizaton of a novel PPTase (encoded by the pptA gene in Figure 13). This PPTase can be used in engineered biosynthesis of polyketides, peptides, hybrid peptide and polyketide metabolites, hybrid polyketide and peptide metabolites, or the combination of both types of metabolites. The PPTase can also be used in converting apo-peptidyl carrier

proteins (both type I and type II) and acyl carrier proteins (both type I and type II) into the holo-proteins.

The Examples provided herein and the accompanying primers permit one of ordinary skill in the art to isolate the *blm* gene cluster of this invention, its constituent ORFs, various modules, or enzymatic domains. The isolated nucleic acid components can be used to express one or more polypeptide components for *in vivo* (*e.g. recombinant)* synthesis of one or more polypeptides and/or polyketides as indicated above. It will also be appreciated that the *blm* cluster polypeptides can be used for *ex vivo* assembly of various macromolecules.

I. BLM gene cluster and the PPTase gene.

A) The BLM gene cluster.

The nucleic acids comprising the *blm* gene cluster are identified in Tables I and II and listed in the sequence listing provided herein (SEQ ID NOS: 1 and 2, GenBank Accession numbers AT-149091, AT-210249, AF210311). In particular, Table I identifies genes and functions of open reading frames (ORFs) responsible for the biosynthesis of the hybrid peptide/polyketide/peptide backbone and sugar moieties of bleomycin, while Table II identifies a number of ORFs comprising the *blm* gene cluster, identifies the activity of the catalytic domain encoded by the ORF and provides primers for the amplification and isolation of that orf.

As illustrated in Example 1, the *blm* cluster comprises a PKS module, flanked by several NRPS modules along with several sugar biosynthesis genes and genes encoding other biosynthesis enzymes as well as several resistance and regulatory genes (Table 1).

Table I. Determined functions of ORFs in the bleomycin biosynthesis gene cluster

Gene	Amino acids	Sequence Homolog[1]	Proposed function[2, 3]
orf8	424	YqeR (BAA12461)	Oxidase
blmC	498	RfaE (AA07904.1)	NDP-glucose synthase
blmI	90	GrsB (P14688)	Type II PCP
blmD	545	NodU (Q53515	Carbamoyl transferase
blmE	390	RfaF (AAD16056)	Glycosyl transferase
orf13	187	MbtH (O05821)	Unknown
blmII	462	Nrp (CAA98937)	NRPS condensation enzyme
orf15	339	SyrP (1890776)	Regulation
blmII	935	HMWP2 (P48633), McbC (P23185)	A PCP Ox

blmIV	2626	HMWP2 (P48633)	C A PCP Cy A PCP Cy
orf18	638	AsnB (2293165)	Asparagine synthetase
blmF	494	RfbC (Q50864)/BlmOrf1 (507319)	Glycosyl transferase/β-hydroxylase
blmG	325	YtcB (2293288)	Sugar epimerase
blmV	645	McyB (2708278)	PCP C
blmVI	2675	ACoAS (1658531), PksD (S73014) SnbDE (CAA67249)	A[4] ACP C A PCP C A
blmVII	1218	SyrE (3510629)	C A PCP
blmVIII	1841	HMWP1 (CAA73127)	KS AT MT KR ACP
blmIX	1066	SafB (1171128)	C A PCP
blmX	2140	TycC (2623773)	C A PCP C A PCP
blmXI	688	SyrE (3510629)	NRPS condensation enzyme
orf28	239	SC9C7.04C (CAA22716)	Unknown
orf29	582	YvdB (CAB08068)	Transmembrane transporter
orf30	113	SmtB (P30340)	Regulation
orf31	117	PhnA (P16680)	Unknown

1. Protein accession numbers are given in parentheses. 2. Underlined domains contain motifs that are clearly different from known NRPS or PKS domains. 3. This A domain lacks the typical NRPS A1, A2, A4, A8, and A9 motifs and more closely resembles acyl CoA synthases. *ORF1* to *ORF7* were reported by Schmidt (1994) *Gene* 151:17-21, who assigned ORF2 as *blmA* and ORF4 as *blmB*.

Noteworthy are the genes encoding the NRPS and PKS enzymes. The *blmI, blmII,* and *blmXI* genes encode NRPSs with an unusual architecture. In contrast to all known NRPSs, which are of modular organization with each module consisting minimally of a condensation (C), an adenylation (A), and a peptidyl carrier protein (PCP) domain, *BlmI, BlmII,* and *BlmXI* are discrete proteins homologous to individual domains of type I NRPSs. We have characterized BlmI as a type II PCP (Du and Shen (1999) *Chem. Biol.* 6: 507-517). The *BlmII* and *BlmXI* proteins can serve as candidates for type II condensation enzymes.

The *blmIII, blmIV, blmV, blmVI, blmVII, blmIX,* and *blmX* genes encode modular NRPSs consisting of domains characteristic for known type I NRPSs, such as the A, PCP, C, and condensation/cyclization (Cy) domains, as well as an unprecedented oxidation (Ox) domain. BlmVI is unique among all the Blm NRPSs identified. Its N-terminal module (NRPS-5) consists of an atypical A domain, which bears a close resemblance to a family of acyl CoA synthases (Fitzmaurice and Kolattukudy (1997) *J. Bacteriol.* 179: 2608-2615; Fitzmaurice and Kolattukudy (1998) *J. Biol. Chem.* 273: 8033-8039), and an acyl carrier protein (ACP)-like domain. Its C-terminal module is truncated and presumably interacts with BlmV to constitute the complete NRPS-3 module (Fig. 1B). Also noteworthy are the C domain of NRPS-3 that lacks both His residues of the conserved HHxxxDG (SEQ ID NO: 4) active site for transpeptidation (Stachelhaus *et al.* (1998) *J. Biol. Chem.* 273: 22773-22781)

and the extra C domain at the C-terminus of BlmV. These unusual features associated with BlmVI and BlmV may play roles in the formation of the β-aminoalaninamide and the pyrimidine moieties of BLM, which are unprecedented in peptide biosynthesis.

The *blmVIII* gene encodes a PKS module consisting of domains characteristic for known PKSs, such as ketoacyl synthase (KS), acyltransferase (AT), ketoreductase (KR), and ACP, with malonyl CoA acting as an extending unit according to sequence comparison of the AT domain (Haydock *et al.* (1995) *FEBS Lett.* 374: 246-248) (Fig. 1B).

The identification of an integrated methyltransferase (MT) domain in the middle of *BlmVIII* is unique, representing the first PKS from actinomycetes that contains an internal MT domain.

Table II. *Blm* gene cluster open reading frames (ORFs) and primers for ORF amplification.

Orf #	Position	Activity	Method	Primers Forward Reverse	Seq ID No.
orf-8	76183-77457	Oxygen-independent coproporphyrinogen III oxidase	Gapped-blast comparison[1]	F: ATGAGCCACGCCATCGGA R: TCAGGCGCGTTCGGGGGC	5 6
orf-9	74690-76186	ADP-heptose synthase (*blmC*)	Gapped-blast comparison[1]	F: GTGAACACCGACCTGCCC R: TCATGGGGTGTCTCCCTC	7 8
orf-10	74421-74693	Peptidyl carrier protein (*blmI*)	Expression and biochemical characterization.[2]	F: ATGAGCGCCCCGCGGGGC R: TCACCGGTCCCGCTCCCC	9 10
orf-11	72787-74424	Carbamyltransferase (*blmD*)	Gapped-blast comparison[1]	F: ATGAGCGCCGACCCGTCC R: TCATGAGCGGGCCGCCGT	11 12
orf-12	71618-72790	ADP-heptose:LPS heptosyl transferase (*blmE*)	Gapped-blast comparison[1]	F: ATGACCACCCCCATGACC R: TCATGGGGTACTCCTGAT	13 14
orf-13	70983-71546	Homolog of mbtH in the synthesis of mycobactin	Gapped-blast comparison[1]	F: ATGACCACGACCCCGCGG R: TCAGGTGCCGGACACGCG	15 16
orf-14	69598-70986	Peptide synthetase (condensation, *blmII*)	Gapped-blast comparison[1]	F: GTGACCGCCCCCGGCACA R: TCATCGGTGGCTCCTCGT	17 18
orf-15	68582-69601	Regulatory gene (homolog of *syrP*)	Gapped-blast comparison[1]	F: GTGAACCGGCACGGCCCC R: TCACGCGCTCACCTCGTC	19 20
orf-16	65778-68585	Mutated peptide synthetase- oxidase (NRPS-0, *blmIII*)	Gapped-blast comparison[1]	F: GTGACGAGCGCCCGGCCC R: TCACGGGGCCTCCGTGCG	21 22
orf-17	57901-65781	Peptide synthetase (NRPS-2-1,*blmIV*)	Expression and biochemical characterization.[2]	F: ATGCTGCACGGCGCCGCG R: TCACTCCGGTCCACCTCC	23 24

orf-18	55899-57815	Asparagine synthetase	Gapped-blast comparison[1]	F: GTGAGGCCCGTGTGCGGC R: TCAGCCACCGTTGCCGCC	25 26
orf-19	54418-55902	Homolog of hydroxylase-dehydrogenase (*blmF*)	Gapped-blast comparison[1]	F: GTGAAGGACCTCGGCCGG R: TCACTCCCCCGGTGCCGG	27 28
orf-20	53427-54404	Nucleotide-sugar epimerase (*blmG*)	Gapped-blast comparison[1]	F: GTGACATGGACCGTGGTG R: TCAGGCATCGGCCCTCCC	29 30
orf-21	51493-53430	Peptide synthetase (NRPS-3CT, *blmV*)	Gapped-blast comparison[1]	F: ATGCGCGGGCATGACGAC R: TCACGGTGTCTCTCCCTC	31 32
orf-22	43263-51290	Peptide synthetase (NRPS-5-4-3, *blmVI*)	Expression and biochemical characterization.[2]	F: ATGAGCCGGCCGGCCGGC R: TCATGCTCGGTCATCGCC	33 34
orf-23	39610-43266	Peptide synthetase (NRPS-6, *blmVII*)	Expression and biochemical characterization.[2]	F: GTGACCACGCCCCGCATC R: TCATTCGGGACGCGGGCA	35 36
orf-24	34088-39613	Polyketide synthase (*blmVIII*)	Gapped-blast comparison[1]	F: ATGAGCCATGCCGACGCG R: TCACAGCACCACCTCTTC	37 38
orf-25	30891-34091	Peptide synthetase (NRPS-7, *blmIX*)	Gapped-blast comparison[1]	F: ATGACCCCGGCCGCCGAC R: TCATCGTCCGCCGCCTTT	39 40
orf-26	24406-30894	Peptide synthetase (NRPS-9-8, *blmX*)	Gapped-blast comparison[1]	F: ATGCCTCGGTGTGCCCGA R: TCATTCGGCGGCACCTCC	41 42
orf-27	22127-24193	Peptide synthetase (condensation, *blmXI*)	Gapped-blast comparison[1]	F: GTGGGTTTCCGTCGAGCG R: TTACACCCTCCGTTTCTC	43 44
orf-28	21367-22086	Phosphatidylserine decarboxylase	Gapped-blast comparison[1]	F: ATGGCACAGGACCTGAAC R: TCAACGCCACCGGATCTT	45 46
orf-29	19161-20909	Transmembrane transporter	Gapped-blast comparison[1]	F: GTGAGCTCCCTCGCCGTC R: TCATCGTCGGGCACTCGG	47 48
orf-30	18823-19164	Metal dependent regulatory element	Gapped-blast comparison[1]	F: GTGCCGGTTCCGCTGTAT R: TCACCGGGCACTGACCTC	49 50
orf-31	18660-18307	PHNA homolog	Gapped-blast comparison[1]	F: GTGACCGAGAACCTTCCG R: TCAGACCTTCTTGACCAC	51 52
orf-32	17736-9211	Peptide synthetase (NRPS-11-10)	Gapped-blast comparison[1]	F: ATGGCCTCAGACGCTTTG R: TCATTGAGACTCCTCCTC	53 54
orf-33	9214-7859	Putative transporter	Gapped-blast comparison[1]	F: ATGATGAAGTCAAGCCGC R: TCAGTGGCTTACAAGGAG	55 56
orf-34	7797-6784	Homolog of clavaminic acid synthase	Gapped-blast comparison[1]	F: ATGACTGACCTGCCGTTG R: TCACACCAGCAGCGAGGT	57 58
orf-35	6773-6021	Thioesterase	Gapped-blast comparison[1]	F: ATGGATTTCCCCCTCACC R: TCATGCCCCTACCTCGGC	59 60
orf-36	6024-4741	Putative transporter	Gapped-blast comparison[1]	F: ATGACCGCGCGCGTCGAC R: TCACTCCTCGGCTTCGGC	61 62
orf-37	4733-3915	Unknown	Gapped-blast comparison[1]	F: GTGTCCAAGAACGCGGCG R: TCATCGGCTCGCCTCGTG	63 64
orf-38	3918-2182	Peptide synthetase (NRPS-12)	Gapped-blast comparison[1]	F: ATGACCCTCACCCTGCGG R: TCACTCGGGCACTCCTTC	65 66
orf-39	2185-1199	Regulatory gene (homolog of *SvrP*	Gapped-blast comparison[1]	F: GTGACCGGTTCCGTAACG R: TCATGAGTCCGCCGAGGT	67 68
orf-	1015-1	Peptide synthetase	Gapped-blast	F: ATGACAGAGGTCCGAGGT	69

40			comparison[1]	R: CCCGGCAACCGCCCTCCC	70
orf-41	On a separate sequence	4'-phosphopantetheinyl transferase (*pptA*)	Expression and biochemical characterization.[2]	F: GTGATCGCCGCCCTCCTG R: TTACGGGACGGCGGTCCG	71 72

The Blm megasynthetase comprises nine NRPS modules and one PKS module forming a hybrid NRPS/PKS/NRPS metasynthetase (Fig. 1A). Inspection of the blm gene cluster (Fig. 2) showed that the Blm NRPS and PKS modules apparently are not organized according to the "colinearity rule" for BLM biosynthesis (Fig. 1). Detailed functional organization of the megasynthetase and the BLM synthetic pathway is provided in Example I.

B) PPTase

This invention also provides the gene (pptA, Fig. 13) encoding phosphopantetheine transferase (PPTase) (GenBank Accession No: AF210311) (*see*, SEQ ID NO: 3). PPTase converts carrier proteins for the growing acyl chain from inactive apo-forms to functional holo-forms by the covalent attachment of the 4'-phosphopantetheine moiety of coenzyme A to a conserved serine residue of the carrier-protein substrate (*see, e.g.*, Fig. 1A).

Using the sequence information provided herein (*e.g.* primer sequences and PPTase sequence) the PPTase nucleic acids can be routinely isolated according to standard methods (*e.g.* PCR amplification). Detailed protocols for the isolation of the PPTase are provided in Example 3.

Other PPTases can be identified using the probes and primers illustrated in Example 3. Briefly, using a primer to the THC motif (5'-C GGC ATG GTC GGC TCC HTN CAN CAY TG -3', SEQ ID NO: 73, where H= C+A, N = A + C + T + G, Y = C + T, K = G + T, R = A + G, W = T + A), and a primer designed around the typical C-terminal PPTase motif (*e.g.*, KEA-1: 5'-T GCA GCA GAA CAG GAG GCK NYC CCA NKG - 3', SEQ ID NO: 74, and KEA-2: 5'- TG GGT CAG CGG GTA CCA NRC YTT RWA - 3', SEQ ID NO: 75), and using *S. verticillus* chromosomal DNA as template, the set of primers THC/KEA-2 a probe can be amplified (about 250 bp), that specifically binds to a PPTase. Libraries of organisms comprising NRPS, PKS, and/or hybrid PKS/NRPS pathways can be probed for the presence of a PPTase sequence. Once hybridizing clones are identified, the PPTase sequence can be isolated according to standard methods well know to those of skill in the art (*see, e.g.*, Example 3).

C) Isolation/preparation of nucleic acids.

In one embodiment, this invention provides nucleic acids for the recombinant expression of a bleomycin. Such nucleic acids include isolated gene cluster(s) comprising open reading frames encoding polypeptides sufficient to direct the assembly of a bleomycin.

In other embodiments of this invention, modified bleomycins (*e.g.* bleomycin analogs), novel polyketides, polypeptides, and combinations thereof (polyketide/polypeptide hybrids) are created by modifying PKSs and/or NRPSs so as to introduce variations into known polymers synthesized by the enzymes. Such variations may be introduced by design, for example to modify a known molecule in a specific way, *e.g.* by replacing a single monomeric unit within a polymer with another, thereby creating a derivative molecule of predicted structure. Alternatively, variations can be made randomly, for example by making a library of molecular variants of a known polymer by systematically or haphazardly replacing one or more modules or enzymatic domains in a known PKS or NRPS with a collection of alternative modules or domains. Production of alternative/modified PKSs, NRPSs and hybrid systems is described below.

Using the primer and sequence information provided herein, one of ordinary skill in the art can routinely isolate/clone the PKS and/or NRPS modules and/or enzymatic domains described herein. For example, the PCR primers provided in Table II, above, can be used to amplify any of the orfs identified therein. Moreover, using the sequence information for the *blm* gene cluster provided herein, the design of other primers suitable of the amplification of individual ORFs, combinations of ORFs, genes, *etc.* is routine.

Typically such amplifications will utilize the DNA of an organism containing the requisite genes (*e.g. Streptomyces verticillus*) as a template. Typical amplification conditions include a PCR mixture consisting of 5 ng of *S verticillus* genomic or plasmid DNA as template, 25 pmoles of ech primers, 25 μM dNTP, 5% DMSO, 2 units of *Taq* polymerase, 1 x buffer, with or without 20% glycerol in a final volume of 50 μL. PCR is carried out (*e.g.* on a Gene Amp PCR System 2400 (Perkin-Elmer/ABI)) with a cycling scheme as follows: initial denaturing at 94°C for 5 min, 24-36 cycles of 45 sec at 94°C, 1 min at 60°C, 2 min at 72°C, followed by additional 7 min at 72°C. One of skill will appreciate that optimization of such a protocol, *e.g.* to improve yield, *etc.* is routine (*see, e.g.*, U.S. Patent No. 4,683,202; Innis (1990) *PCR Protocols A Guide to Methods and Applications* Academic Press Inc. San Diego, CA, *etc*). In addition, primer may be designed to introduce restriction sites and so facilitate cloning of the amplified sequence into a vector.

Using the information provided herein other approaches to cloning the desired sequences will be apparent to those of skill in the art. For example, the PKS or NRPS modules or enzymatic domains of interest can be obtained from an organism that expresses the same, using recombinant methods, such as by screening cDNA or genomic libraries, derived from cells expressing the gene, or by deriving the gene from a vector known to include the same. The gene can then be isolated and combined with other desired NRPS and/or PKS modules or domains, using standard techniques. If the gene in question is already present in a suitable expression vector, it can be combined *in situ,* with, e.g., other PKS subunits, as desired. The gene of interest can also be produced synthetically, rather than cloned. The nucleotide sequence can be designed with the appropriate codons for the particular amino acid sequence desired. In general, one will select preferred codons for the intended host in which the sequence will be expressed. The complete sequence can be assembled from overlapping oligonucleotides prepared by standard methods and assembled into a complete coding sequence (*see, e.g.,* Edge (1981) *Nature* 292:756; Nambair *et al.* (1984) *Science* 223: 1299; Jay *et al.* (1984) *J. Biol. Chem.* 259:6311). In addition, it is noted that custom gene synthesis is commercially available (*see, e.g.* Operon Technologies, Alameda, CA).

Examples of such techniques and instructions sufficient to direct persons of skill through many cloning exercises are found in Berger and Kimmel (1989) *Guide to Molecular Cloning Techniques, Methods in Enzymology 152* Academic Press, Inc., San Diego, CA (Berger); Sambrook *et al.* (1989) *Molecular Cloning - A Laboratory Manual* (2nd ed.) Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor Press, NY; Ausubel (19 1994) *Current Protocols in Molecular Biology,* Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., U.S. Patent 5,017,478; and European Patent No. 0,246,864.

II. Expression of blm gene clusters, modules, and enzymatic domains.

As indicated above, in one embodiment this invention provides novel NRPS and PKS genes for the efficient recombinant production of both novel and known polyketides, peptides, and polyketide/polypeptide hybrids by expressing them *in vivo.* In other embodiments, such syntheses are carried out *in vitro.* Even *in vitro* syntheses, however, typically utilize recombinantly expressed PKSs, NRPSs, or enzymatic domains thereof. Thus, it is frequently desirable to express protein components of the PKSs or NRPs described above.

Typically expression of the protein components of the pathway and/or of the products of the NRPS/PKS pathway is accomplished by placing the subject PKS or NRPS nucleic acid(s) in an expression vector, and transfecting a cell with the vector such that the cell expresses the desired product(s).

A) Expression vectors

The choice of vector depends on the sequence(s) that are to be expressed. Any transducible cloning vector can be used as a cloning vector for the nucleic acid constructs of this invention. However, where large clusters are to be expressed, it phagemids, cosmids, P1s, YACs, BACs, PACs, HACs or similar cloning vectors be used for cloning the nucleotide sequences into the host cell. Phagemids, cosmids, and BACs, for example, are advantageous vectors due to the ability to insert and stably propagate therein larger fragments of DNA than in M13 phage and lambda phage, respectively. Phagemids which will find use in this method generally include hybrids between plasmids and filamentous phage cloning vehicles. Cosmids which will find use in this method generally include lambda phage-based vectors into which cos sites have been inserted. Recipient pool cloning vectors can be any suitable plasmid. The cloning vectors into which pools of mutants are inserted may be identical or may be constructed to harbor and express different genetic markers (see, *e.g.*, Sambrook *et al.*, *supra*). The utility of employing such vectors having different marker genes may be exploited to facilitate a determination of successful transduction.

In preferred embodiments of this invention, vectors are used to introduce PKS, NRPS, or NRPS/PKS genes or gene clusters into host (*e.g. Streptomyces*) cells. Numerous vectors for use in particular host cells are well known to those of skill in the art. For example described in Malpartida and Hopwook, (1984) *Nature*, 309:462-464; Kao *et al.*, (1994), *Science*, 265: 509-512; and Hopwood *et al.*, (1987) *Methods Enzymol.*, 153:116-166 all describe vectors for use in various *Streptomyces* hosts.

In a preferred embodiment, *Streptomyces* vectors are used that include sequences that allow their introduction and maintenance in *E. coli*. Such *Streptomyces/E. coli* shuttle vectors have been described (*see*, for example, Vara *et al.*, (1989) *J. Bacteriol.*, 171:5872-5881; Guilfoile & Hutchinson (1991) *Proc. Natl. Acad. Sci. USA*, 88: 8553-8557.)

The gene sequences, or fragments thereof, which collectively encode a PKS and/or NRPS and/or PKS/NRPS gene cluster, one or more ORFs, one or more modules, or one or more enzymatic domains of this invention, can be inserted into one or more

expression vectors, using methods known to those of skill in the art. Expression vectors will include control sequences operably linked to the desired NRPS and/or PKS coding sequence or fragment thereof. Suitable expression systems for use with the present invention include systems that function in eucaryotic and prokaryotic host cells. However, as explained above, prokaryotic systems are preferred, and in particular, systems compatible with *Streptomyces spp.* are of particular interest. Control elements for use in such systems include promoters, optionally containing operator sequences, and ribosome binding sites. Particularly useful promoters include control sequences derived from PKS and/or NRPS gene clusters, such as one or more *act* promoters. However, other bacterial promoters, such as those derived from sugar metabolizing enzymes, such as galactose, lactose (*lac*) and maltose, will also find use in the present constructs. Additional examples include promoter sequences derived from biosynthetic enzymes such as tryptophan (*trp*), the beta -lactamase (*bla*) promoter system, bacteriophage lambda PL, and T5. In addition, synthetic promoters, such as the tac promoter (U.S. Patent 4,551,433), which do not occur in nature also function in bacterial host cells. In *Streptomyces*, numerous promoters have been described including constitutive promoters, such as *ermE* and *tcmG* (Shen and Hutchinson, (1994) *J. Biol. Chem.* 269: 30726-30733), as well as controllable promoters such as *actI* and *actIII* (Pieper *et al.*, (1995) *Nature*, 378: 263-266; Pieper *et al.*, (1995) *J. Am. Chem. Soc.*, 117: 11373-11374; and Wiesmann *et al.*, (1995) *Chem. & Biol.* 2: 583-589).

Other regulatory sequences may also be desirable which allow for regulation of expression of the PKS replacement sequences relative to the growth of the host cell. Regulatory sequences are known to those of skill in the art, and examples include those which cause the expression of a gene to be turned on or off in response to a chemical or physical stimulus, including the presence of a regulatory compound. Other types of regulatory elements may also be present in the vector, for example, enhancer sequences.

Selectable markers can also be included in the recombinant expression vectors. A variety of markers are known which are useful in selecting for transformed cell lines and generally comprise a gene whose expression confers a selectable phenotype on transformed cells when the cells are grown in an appropriate selective medium. Such markers include, for example, genes that confer antibiotic resistance or sensitivity to the plasmid. Alternatively, several polyketides are naturally colored and this characteristic provides a built-in marker for selecting cells successfully transformed by the present constructs.

The various PKS and/or NRPS clusters or subunits of interest can be cloned into one or more recombinant vectors as individual cassettes, with separate control elements, or under the control of, *e.g.*, a single promoter. The PKS and/or NRPS subunits can include flanking restriction sites to allow for the easy deletion and insertion of other PKS subunits so that hybrid PKSs can be generated. The design of such unique restriction sites is known to those of skill in the art and can be accomplished using the techniques described above, such as site-directed mutagenesis and PCR.

Methods of cloning and expressing large nucleic acids such as gene clusters, including PKS- or NRPS-encoding gene clusters, in cells including *Streptomyces* are well known to those of skill in the art (*see, e.g.*, Stutzman-Engwall and Hutchinson (1989) *Proc. Natl. Acad. Sci. USA*, 86: 3135-3139; Motamedi and Hutchinson (1987) *Proc. Natl. Acad. Sci. USA*, 84: 4445-4449; Grim *et al.* (1994) *Gene*, 151: 1-10; Kao *et al.* (1994) *Science*, 265: 509-512; and Hopwood *et al.* (1987) *Meth. Enzymol.*, 153: 116-166). In some examples, nucleic acid sequences of well over 100kb have been introduced into cells, including prokaryotic cells, using vector-based methods (*see*, for example, Osoegawa *et al.*, (1998) *Genomics*, 52: 1-8; Woon *et al.*, (1998) *Genomics*, 50: 306-316; Huang *et al.*, (1996) *Nucl. Acids Res.*, 24: 4202-4209). In addition, the cloning and overexpression of NRPS-1 and NRPS-6 is illustrated in Example 1.

In certain embodiments this invention may make use of genetically engineered cells that either lack PKS and/or NRPS genes or have their naturally occurring PKS and/or NRPS genes substantially deleted. These host cells can be transformed with recombinant vectors, encoding a variety of PKS and/or NRPS gene clusters, for the production of active polyketides. The invention provides for the production of significant quantities of product, *e.g.* a bleomycin, at an appropriate stage of the growth cycle. The BLMs or other hybrid polyketide/peptide metabolites so produced can be used as therapeutic agents, to treat a number of disorders, depending on the type of metabolites in question. For example, several of the polyketides and peptides produced by the present method will find use as immunosuppressants, as anti-tumor agents, as well as for the treatment of viral, bacterial and parasitic infections. The ability to recombinantly produce polyketides and peptides also provides a powerful tool for characterizing PKSs and/or NRPSs and the mechanism of their actions.

B) Host cells.

The vectors described above can be used to express various protein components of the polyketide and/or polypeptide synthetic modules for subsequent isolation and/or to provide a biological synthesis of one or more desired biomolecules (*e.g* polyketides, peptides, *etc.*). Where one or more proteins of the *blm* cluster are expressed (*e.g.* overexpressed) for subsequent isolation and/or characterization, the proteins are expressed in any prokaryotic or eukaryotic cell suitable for protein expression. In one preferred embodiment, the proteins are expressed in *E. coli.* Overexpression of *blmI* in *E. coli* is described in Example 2.

Host cells for the recombinant production of the subject polyketides can be derived from any organism with the capability of harboring a recombinant PKS, NRPS or PKS/NRPS gene cluster. Thus, the host cells of the present invention can be derived from either prokaryotic or eucaryotic organisms. However, preferred host cells are those constructed from the actinomycetes, a class of mycelial bacteria which are abundant producers of a number of polyketides and peptides. A particularly preferred genus for use with the present system is Streptomyces. Thus, for example, *S. verticillus S. ambofaciens, S. avermitilis, S. azureus, S. cinnamonensis, S. coelicolor, S. curacoi, S. erythraeus, S. fradiae, S. galilaeus, S. glaucescens, S. hygroscopicus, S. lividans, S. parvulus, S. peucetius, S. rimosus, S. roseofulvus, S. thermotolerans, S. violaceoruber*, among others, will provide convenient host cells for the subject invention, with *S. coelicolor* being preferred (*see, e.g., Hopwood, D. A. and Sherman, D. H. Ann. Rev. Genet. (1990) 24:37-66; O'Hagan, D. The Polyketide Metabolites (Ellis Horwood Limited, 1991), for a description of various polyketide-producing organisms and their natural products.)*

In a preferred embodiment, the above-described cells are genetically engineered by deleting one or more naturally occurring PKS and/or NRPS genes therefrom, using standard techniques, such as by homologous recombination. (*see, e.g.*, Khosla, *et al.* (1992) *Molec. Microbiol.* 6: 3237).

In certain embodiments, a eukaryotic host cell is preferred (*e.g.* where certain glycosylation patterns are desired). Suitable eukaryotic host cells are well known to those of skill in the art. Such eukaryotic cells include, but are not limited to yeast cells, insect cells, plant cells, fungal cells, and various mammalian cells (*e.g.* COS, CHO HeLa cells lines and various myeloma cell lines)

C) Protein/polyketide recovery.

Polypeptide and/or polyketide recovery is accomplished according to standard methods well known to those of skill in the art. Thus, for example where *blm* cluster proteins are to be expressed and isolated, the proteins can be expressed with a convenient tag to facilitate isolation (*e.g.* a His_6) tag. Other standard protein purification techniques are suitable and well known to those of skill in the art (*see, e.g.*, Quadri *et al.* (1998) *Biochemistry* 37: 1585-1595; Nakano *et al.* (1992) *Mol. Gen. Genet.* 232: 313-321, *etc.*).

Similarly where components (*e.g.* modules and/or enzymatic domains) of the *blm* cluster are used to express various biomolecules (*e.g.* polyketides, sugars, polypeptides, *etc.*) the desired product and/or shunt metabolite(s) are isolated according to standard methods well know to those of skill in the art (*see, e.g.*, Carreras and Khosla (1998) *supra.*) Purification and in vitro reconstitution of the essential protein components of an aromatic polyketide synthase. *Biochemistry* 37: 2084-2088, Deutscher (1990) *Methods in Enzymology Volume 182: Guide to Protein Purification*, M. Deutscher, ed. .

III. Synthesis of recombinant bleomycins.

In one embodiment this invention provides methods of synthesizing bleomycins and recombinantly synthesized bleomycins. As indicated above, this is generally accomplished by providing an organism (*e.g.* a bacterial cell) containing sufficient compoents of the *blm* gene cluster to direct synthesis of a complete bleomycin.

In one embodiment, the entire *blm* cluster is cloned into a *Streptomyces* strain (*e.g., S. lividans* or *S. coelicolor*). Kao *et al.*(1994) *Science,* 265: 509-512, have cloned the 30 kb DEBS genes from *Sacc. erythmea* into *S. coelicolor* and produced 6-deoxyerythronolide B in *S. coelicolor* and these methods can be used construct an expression plasmid for heterologous expression of the *blm* cluster. This method involves the transfer of DNA between a temperature-sensitive plasmid and a shuttle vector by means of a homologous double recombination event in *E. coli* (*Id.*). In a preferred embodiment, the two ends spanning the *blm* cluster are cloned into a temperature-sensitive plasmid that is chloramphenicol resistant (CM^R) such as pCK6. *S. verticillus* DNA is then rescued from a donor into the temperature-sensitive recipient by co-transforming *E. coli* with the Cm^R recipient plasmid and the apramycin resistant (Ap^R) pKC505 donor cosmid that contains the *blm* gene cluster, followed by chloramphenicol and apramycin selection at 30°C. Colonies harboring both plasmids (Cm^R, Ap^R) will be shifted to 44°C on chloramphenicol and apramycin plates and only those cointegrates formed by a single recombination event

between the two plasmids are viable. Surviving colonies are then propagated at 30°C on Cm^R plates to select for recombinant plasmids formed by the resolution of cointegrates through a second recombinant event. The desired *blm* cluster is cloned into the Cm^R temperature-sensitive plasmid and is ready to be moved into any expression plasmid by a similar means of homologous recombinant event.

For example, if pWHM861 is the choice of shuttle plasmid for the expression of the *blm* cluster in *S. lividans* (Meurer and Hutchinson (1995) *J. Bacteriol.*, 177: 477-481), the two ends spanning the *blm* cluster downstream of the *ErmE** promoter in the ampicillin resistant (AM^R) plasmid pWHM861 are cloned. The resulting plasmid is co-transformed with the temperature-sensitive plasmid containing the *blm* cluster described above into *E. coli* under the selection of chloramphenicol and ampicillin at 30°C. These Cm^R and AM^R colonies are shifted to 44°C on chloramphenicol and ampicillin plates to undergo a single recombination event and the surviving colonies are resolved on ampicillin plates at 30°C by completing the double recombination process. The resulting plasmid is suitable for transformation into *S. lividans* by selection of thiostrepton, in which the expression of the desired *blm* cluster is under the control of the *ErmE** promoter. The *S. lividans* transformants are cultured and any metabolites produced are isolated and characterized.

Once production of BLM in *S. lividans* is established, mutated alleles of the *blm* synthetase can be introduced into the *blm* cluster for the production of BLM analogs.

IV. Altered endogenous expression of bleomycins.

Using the Blm gene cluster information provided herein, one of skill in the art may regulating the synthesis of endogenous bleomycin. The expression of various ORFs comprising the *blm* gene cluster may be increased or decreased to alter bleomycin synthesis levels.

Methods of altering the expression of endogenous genes are well known to those of skill in the art. Typically such methods involve altering or replacing all or a portion of the regulatory sequences controlling expression of the particular gene that is to be regulated. In a preferred embodiment, the regulatory sequences (*e.g.*, the native promoter) upstream of one or more of the *blm* ORFs are altered.

This is typically accomplished by the use of homologous recombination to introduce a heterologous nucleic acid into the native regulatory sequences. To downregulate expression of one or more *blm* ORFs, simple mutations that either alter the reading frame or disrupt the promoter are suitable. To upregulate expression of the *blm* ORF(s) the native

promoter(s) can be substituted with heterologous promoter(s) that induce higher than normal levels of transcription.

In a particularly preferred embodiment, nucleic acid sequences comprising the structural gene in question or upstream sequences are utilized for targeting heterologous recombination constructs.

The use of homologous recombination to alter expression of endogenous genes is described in detail in U.S. Patent 5,272,071, WO 91/09955, WO 93/09222, WO 96/29411, WO 95/31560, and WO 91/12650.

V. Synthesis of BLM analogs.

In one one embodiment, this invention provides methods of synthesizing modified bleomycins or bleomycin analogs. In preferred embodiments, the BLM analogs are synthesized either by introducing specific perturbations into individual NRPS and/or PKS enzymatic domains or modules, or by reprogramming the linear order in which the NRPS or PKS enzymatic domains and/or modules appear in the *blm* synthetase genes. The former will lead to BLM analogs with targeted modifications at the BLM backbone and the latter will allow incorporation of other extension units in variable sequence into the biosynthesis of BLM. In particularly preferred embodiments, the genetically modified *blm* synthetases are produced in *S. verticilus*, however, it will be recognized that the entire *blm* gene cluster can be cloned into other hosts, *e.g.* into *S. lividans* or *S. coelicolor.*

In preferred embodiments modification of the *blm* gene cluster to yield BLM analogues is accomplished by one of two different approaches. In one approach, the BLM enzymatic domains and/or modules modules are altered in a directed manner (*i.e.* they are changed in a preselected way), while in another approach, random/haphazard alterations are introduced into the *blm* cluster and the resulting products are screened to identify those with desired properties.

A) Synthesis of BLM analogs by specific engineering of the *blm* synthetase genes.

The *blm* synthetase genes can be re-engineered by means of specific mutations or by reprogramming the linear order of the NRPS or PKS enzymatic domains or modules. In this approach, a wild-type *blm* synthetase allele is replaced with these mutants in and expressed in an appropriate host (*e.g., S. verticillus* or in a heterologous host). Since both NRPSs (Stachelhaus *et al.* (1995) *Science*, 269: 69-72) and PKSs (Donadio *et al.* (1993)

Proc. Natl. Acad. Sci. USA, 90: 7119-7123, Donadio *et al.* (1995) *J. Am., Chem. Soc.*, 117: 9105-9106, Cortes *et al.* (1995) *Science*, 268: 1487-1489) have shown considerable tolerance to reprogramming, it is expected that these modifications of the BLM synthetase will result in the production of BLM analogs with predicted structural alterations. For example, targeted modification at the (2S,3S,4R)-4-amino-3-hydroxy-2-methyl/pentanoic acid AHM moiety of BLM can be accomplished by introduction of mutations into the *BLMVIII* PKS module of the BLM synthetase locus. Inactivation of the MT or KR motif by in-frame deletion or site-directed mutagenesis will result in the production of BLM analogs containing a demethyl-AHM, oxo-AHM, or oxo-demethyl-AHM moiety, *etc.*

Alternatively, individual functional NRPS domains and/or the PKS module can be deleted or the PKS module can be duplicated in-frame to produce BLM analogs with shorter or longer backbone, respectively. Alternatively, or in addition, the NRPS domains or the PKS module can be rearranged for the production of BLM analogs with a completely different backbone. The NRPS and PKS features can be combined into one integrated system, providing access to a structural variation not available by either the NRPS or PKS system alone.

To create such mutations, plasmids are constructed carrying in-frame deletions of DNA segments encompassing a portion of the *blm* synthetase activities. Construction of specific deletions is preferably accomplished by one of the following two strategies. The first involves subcloning of a DNA fragment in a gene replacement vector, selection of two restriction sites suitably located at the two ends of the DNA segments, and deletion of this segment from within the plasmid by rejoining the two resulting ends. An in-frame deletion can be obtained by a suitable combination of Klenow filling and S1 treatment of both ends prior to ligation.

The second approach involves polymerase chain reaction (PCR) amplification of two DNA segments that separate the region to be deleted followed by joining of the two fragments in the correct orientation in a gene replacement vector. This can be accomplished by designing PCR primers with suitable restriction sites. The restriction site used to generate the deletion and the sequences to serve as templates for the PCR amplification are chosen so as to generate two segments of *blm* synthetase DNA of approximately equal length in the construction in order to maximize the chance of gene replacement. The gene replacement vector containing the allelic or deletion mutation is introduced into a *Streptomyces* strain (*e.g., S. verticillus*). Integration of the plasmid into the *S. verticillus* chromosome via a single reciprocal homologous recombination will yield a recombinant that will be isolated by

selection for the vector marker. The resulting integrants are then grown under non-selective conditions and further resolution by selection for the loss of the vector marker via the second homologous recombination event will produce the desired deletion mutants.

Southern analysis of the isolated deletion mutants with the target DNA is performed to ensure that the expected double crossover recombination event has taken place. The first approach is convenient if there are suitably spaced restriction sites in the DNA sequence. The second approach enables the deletion of any DNA segment but may be limited by the size of the DNA segments that can be amplified by PCR. These *S. verticillus* recombinants are cultured under typical conditions for BLM production and the fermentation broth is screened for the production of any novel BLM analogs resulted from the specific mutations in the *blm* synthetase locus.

B) Synthesis of BLM analogs by "random" modification of *blm* synthetase genes.

Bleomycin analogs can also be synthesized by randomly/haphazardly altering genes in the BLM cluster expressing the products of the randomly modified megasynthetase and then screening the products for the desired activity. Methods of "randomly" altering *blm* cluster genes are described below.

VI. Generation of other synthetic systems.

In addition to the production of bleomycin or modified bleomycins, the *blm* gene cluster or elements thereof can be used by themselves or in combination with NRPS and/or PKS modules and/or enzymatic domains of other PKS and/or NRPS systems to produce a wide variety of compounds including, but not limited to various polyketides, polypeptides, polyketide/polypeptide hybrids, various oxazoles and thiazoles, various sugars, various methylated polypeptides/polyketides, and the like. As with the production of modified bleomycins described above, such compounds can be produced, *in vivo* or *in vitro*, by catalytic biosynthesis using large, modular PKSs, NRPSs, and hybrid PKS/NRPS systems. The megasynthetases directing such syntheses can be rationally designed *e.g.* by predetermined alteration/modification of polyketide and/or polypeptide and/or hybrid PKS/NRPS pathways. Alternatively, large combinatorial libraries of cells harboring various megasynthetases can be produced by the random modification of particular pathways and then selected for the production of a molecule or molecules of interest. It will be appreciated that, in certain embodiments, such libraries of megasynthetases/modified pathways, can be

used to generate large, complex combinatorial libraries of compounds which themselves can be screened for a desired activity.

A) Directed modification of biom lecules.

Elements (*e.g.* open reading frames) of the *blm* biosynthetic gene cluster and/or variants thereof can be used in a wide variety of "directed" biosynthetic processes (*i.e.* where the process is designed to modify and/or synthesize one or more particular preselected metabolite(s)). Polypepitdes encoded by particular open reading frames or combinations of open reading frames can be utilized to perform particular chemical modifications of biological molecules.

Thus, for example, open reading frames encoding a polypeptide synetase can be used to chemically modify an amino acid by coupling it to another amino acid. In another example, the methyl transferase in *BlmVIII* can be utilized to introduce methyl groups into polyketides, and other, substrates. The glycosyl transferases can be used to glycosylate appropriate substrates, and so forth. These examples, are merely illustrative. One of skill in the art, utilizing the information provided here, can perform literally countless chemical modifications and/or syntheses using either "native" bleomycin biosynthesis metabolites as the substrate molecule, or other molecules capable of acting as substrates for the particular enzymes in question. Other substrates can be identified by routine screening. Methods of screening enzymes for specific activity against particular substrates are well known to those of skill in the art.

The biosyntheses can be performed *in vivo*, *e.g.* by providing a host cell comprising the desired *blm* gene cluster open reading frame(s) and/or *in vivo*, *e.g.*, by providing the polypeptides encoded by the *blm* gene cluster ORFs and the appropriate substrates and/or cofactors.

B) Directed engineering of novel synthetic pathways.

In numerous embodiments of this invention, novel polyketides, polypeptides, and combinations thereof are created by modifying known PKSs or NRPSs so as to introduce variations into known polymers synthesized by the enzymes. Such variations may be introduced by design, for example to modify a known molecule in a specific way, *e.g.* by replacing a single monomeric unit within a polymer with another, thereby creating a derivative molecule of predicted structure. Such variations can also be made by adding one or more modules to a known PKS or NRPS, or by removing one or more module from a

known PKS or NRPS. Such novel PKSs or NRPSs can readily be made using a variety of techniques, including recombinant methods and *in vitro* synthetic methods.

Using any of these methods, it is possible to introduce PKS domains into a NRPS, or vice versa, thereby creating novel molecules including both peptide and polyketide structural domains. For example, a PKS enzyme producing a known polyketide can be modified so as to include an additional module that adds a peptide moiety into the polyketide. Novel molecules synthesized using these methods can be screened, using standard methods, for any activity of interest, such as antibiotic activity, effects on the cell cycle, effects on the cytoskeleton, etc.

Novel polyketides, polypeptides, or combinations thereof can also be made by creating novel PKSs or NRPSs *de novo*, using recombinant or *in vitro* synthetic methods. Such novel arrangements of domains can be designed, *i.e.* to create a specific polymer. In addition to creating novel PKSs or NRPSs by combining modules, the methods of this invention can also be used to make novel modules that can add new monomeric units to a growing polypeptide or polyketide chain. Because the identity of each module, and, consequently, the identity of the monomer added by the module, is determined by the identity and number of the functional domains comprising the module, it is possible to produce novel monomeric units by creating novel combinations of functional domains within a module. Such novel modules can be created by design, for example to make a specific module that will add a specific monomer to a polyketide or polypeptide, or can be created by the random association of domains so as to produce libraries of novel modules. Such novel modules can be made using recombinant or *in vitro* synthetic means.

Mutations can be made to the native NRPS and/or PKS subunit sequences and such mutants used in place of the native sequence, so long as the mutants are able to function with other PKS and/or PKS subunits to collectively catalyze the synthesis of an identifiable polyketide and/or polypeptide. Such mutations can be made to the native sequences using conventional techniques such as by preparing synthetic oligonucleotides including the mutations and inserting the mutated sequence into the gene encoding a NRPS and/or PKS subunit using restriction endonuclease digestion. (*see, e.g.,* Kunkel, (1985) *Proc. Natl. Acad. Sci. USA* 82: 448; Geisselsoder *et al.* (1987) *BioTechniques* 5: 786). Alternatively, the mutations can be effected using a mismatched primer (generally 10-20 nucleotides in length) which hybridizes to the native nucleotide sequence, at a temperature below the melting temperature of the mismatched duplex. The primer can be made specific by keeping primer length and base composition within relatively narrow limits and by keeping the mutant base

centrally located (Zoller and Smith (1983) *Meth. Enzymol.* 100: 468). Primer extension is effected using DNA polymerase, the product cloned and clones containing the mutated DNA, derived by segregation of the primer extended strand, selected. Selection can be accomplished using the mutant primer as a hybridization probe. The technique is also applicable for generating multiple point mutations (*see, e.g.*, Dalbie-McFarland *et al.* (1982) *Proc. Natl. Acad. Sci USA* 79:6409). PCR mutagenesis will also find use for effecting the desired mutations.

C) Random modification of PKS/NRPS pathways.

In another embodiment, variations can be made randomly, for example by making a library of molecular variants of a known polymer by randomly mutating one or more PKS or NRPS modules and/or enzymatic domains or by randomly replacing one or more modules or enzymatic domains in a known PKS or NRPS with a collection of alternative modules and/or enzymatic domains..

The PKS and/or NRPS modules can be combined into a single multi-modular enzyme, thereby dramatically increasing the number of possible combinations obtained using these methods. These combinations can be made using standard recombinant or nucleic acid amplification methods, for example by shuffling nucleic acid sequences encoding various modules or enzymatic domains to create novel arrangements of the sequences, analogous to DNA shuffling methods described in Crameri *et al.*, (1998) Nature 391: 288-291, and in U.S. Patents 5,605,793 and in 5,837,458. In addition, novel combinations can be made *in vitro*, for example by combinatorial synthetic methods. Novel polymers, or polymer libraries, can be screened for any specific activity using standard methods.

Random mutagenesis of the nucleotide sequences obtained as described above can be accomplished by several different techniques known in the art, such as by altering sequences within restriction endonuclease sites, inserting an oligonucleotide linker randomly into a plasmid, by irradiation with X-rays or ultraviolet light, by incorporating incorrect nucleotides during in vitro DNA synthesis, by error-prone PCR mutagenesis, by preparing synthetic mutants or by damaging plasmid DNA in vitro with chemicals. Chemical mutagens include, for example, sodium bisulfite, nitrous acid, hydroxylamine, agents which damage or remove bases thereby preventing normal base-pairing such as hydrazine or formic acid, analogues of nucleotide precursors such as nitrosoguanidine, 5-bromouracil, 2-aminopurine, or acridine intercalating agents such as proflavine, acriflavine, quinacrine, and the like.

Generally, plasmid DNA or DNA fragments are treated with chemicals, transformed into *E. coli* and propagated as a pool or library of mutant plasmids.

Large populations of random enzyme variants can be constructed *in vivo* using "recombination-enhanced mutagenesis." This method employs two or more pools of, for example, 10^6 mutants each of the wild-type encoding nucleotide sequence that are generated using any convenient mutagenesis technique, described more fully above, and then inserted into cloning vectors.

D) Incorporation and/or modification of non-blm cluster elements.

In either the directed or random approaches, nucleic acids encoding novel combinations of modules and/or enzymatic are introduced into a cell. In one embodiment, nucleic acids encoding one or more PKS or NRPS domains are introduced into a cell so as to replace one or more domains of an endogenous PKS or NRPS within a chromosome of the cell. Endogenous gene replacement can be accomplished using standard methods, such as homologous recombination. Nucleic acids encoding an entire PKS, NRPS, or combination thereof can also be introduced into a cell so as to enable the cell to produce the novel enzyme, and, consequently, synthesize the novel polymer. In a preferred embodiment, such nucleic acids are introduced into the cell optionally along with a number of additional genes, together called a 'gene cluster,' that influence the expression of the genes, survival of the expressing cells, *etc.* In a particularly preferred embodiment, such cells do not have any other PKS- or NRPS- encoding genes or gene clusters, thereby allowing the straightforward isolation of the polymer synthesized by the genes introduced into the cell.

Furthermore, the recombinant vector(s) can include genes from a single PKS and/or NRPS gene cluster, or may comprise hybrid replacement PKS gene clusters with, *e.g.*, a gene for one cluster replaced by the corresponding gene from another gene cluster. For example, it has been found that ACPs are readily interchangeable among different synthases without an effect on product structure. Furthermore, a given KR can recognize and reduce polyketide chains of different chain lengths. Accordingly, these genes are freely interchangeable in the constructs described herein. Thus, the replacement clusters of the present invention can be derived from any combination of PKS and/or NRPS gene sets that ultimately function to produce an identifiable polyketide and/or peptide.

Examples of hybrid replacement clusters include, but are not limited to, clusters with genes derived from two or more of the *act* gene cluster, the *whiE* gene cluster, frenolicin (*fren*), granaticin (*gra*), tetracenomycin (*tcm*), 6-methylsalicylic acid (6-msas),

oxytetracycline (*otc*), tetracycline (*tet*), erythromycin (*ery*), griseusin (*gris*), nanaomycin, medermycin, daunorubicin, tylosin, carbomycin, spiramycin, avermectin, monensin, nonactin, curamycin, rifamycin and candicidin synthase gene clusters, among others. (For a discussion of various PKSs, *see, e.g.*, Hopwood and Sherman (1990) *Ann. Rev. Genet.* 24: 37-66; O'Hagan (1991) The Polyketide Metabolites, Ellis Horwood Limited.

A number of hybrid gene clusters have been constructed, having components derived from the *act, fren, tcm, gris* and *gra* gene clusters (*see, e.g.*, U.S. Patent 5,712,146). Other hybrid gene clusters, as described above, can easily be produced and screened using the disclosure herein, for the production of identifiable polyketides, polypeptides or polyketide/polypeptide hybrids.

Host cells (*e.g. Streptomyces*) can be transformed with one or more vectors, collectively encoding a functional PKS/NRPS set (*e.g.* a bleomycin or bleomycin analog), or a cocktail comprising a random assortment of PKS and/or NRPS genes, modules, active sites, or portions thereof. The vector(s) can include native or hybrid combinations of PKS and/or NRPS subunits or cocktail components, or mutants thereof. As explained above, the gene cluster need not correspond to the complete native gene cluster but need only encode the necessary PKS and/or NRPS components to catalyze the production of the desired product. For example, in *Streptomyces* aromatic PKSs, carbon chain assembly requires the products of three open reading frames (ORFs). ORF1 encodes a ketosynthase (KS) and an acyltransferase (AT) active site (KS/AT); ORF2 encodes a chain length determining factor (CLF), a protein similar to the ORF1 product but lacking the KS and AT motifs; and ORF3 encodes a discrete acyl carrier protein (ACP). Some gene clusters also code for a ketoreductase (KR) and a cyclase, involved in cyclization of the nascent polyketide backbone. However, it has been found that only the KS/AT, CLF, and ACP, need be present in order to produce an identifiable polyketide. Thus, in the case of aromatic PKSs derived from *Streptomyces*, these three genes, without the other components of the native clusters, can be included in one or more recombinant vectors, to constitute a "minimal" replacement PKS gene cluster.

E) Variation of starter and extender units.

In addition to varying the PKS and/or NRPS modules and/or domains, variations in the products produced by various PKS/NRPS systems can be obtained by varying the starter units and/or the extender units. Thus, for example, a considerable degree of variability exists for starter units, *e.g.*, acetyl CoA, maloamyl CoA, propionyl CoA,

acetate, butyrate, isobutyrate and the like. In addition, naturally occurring PKSs and/or NRPSs have shown some tolerance for varying extender units.

F) Examples of preferred modifications.

As indicated above, the novel PKS and NRPS modules and enzymatic domains identified herein can be used to perform specific single modifications of particular substrates, or as components of complex synthetic pathways to generate particular products or large combinatorial libraries. As described in the Examples, a number of modules of the *blm* gene cluster provide novel functionality. By way of example, a few preferred reactions are listed below. These examples are intended to be illustrative and are not exhaustive nor limiting.

1. Use of *BlmVIII* PKS to introduce branched methyl group.

The *blmVIII* gene identified herein encodes a PKS module consisting of domains characteristic for known PKSs, such as ketoacyl synthase (KS), acyltransferase (AT), ketoreductase (KR), and ACP, with malonyl CoA acting as an extending unit. However, the identification of an integrated methyltransferase (MT) domain in the middle of *BlmVIII* is unique, representing the first PKS from actinomycetes that contains an internal MT domain. The use of this methyltransferase domain allows the introduction of a branched methyl group during a polyketide and/or polypeptide and/or hybriding polyketide/polypeptide synthesis. Figure 5 illustrates the use of *BlmVIII* PKS in engineering a polyketide biosynthesis that introduces a branched methyl group.

The first formula in Figure 5 illustrates a polyketide synthesis mediated by 6-deoxyerythronolide B synthase (DEBS) which normally catalyzes the biosynthesis of the erythromycin aglycone, 6-deoxyerythronolide B. The remaining formulas show how the use of the *blmVIII* methyltransferase (MT) group at different points in the synthesis results in the introduction of a methyl group at different locations in the resulting product.

In view of this illustration, one of skill in the art would appreciate that the *blmVIII* MT domain can be used in a wide variety of biosyntheses to introduce methyl branches.

2. Use of the *blm* gene cluster to make thiazolidine, thiazoline, thiazole, bi-thiazolidine, bithiazoline, and bithiazole-containing compounds.

The *BlmIV* and *BlmIII* NRPSs are characterized by unusual Cy domains as well an unprecedented Ox domain, providing an efficient biosynthesis for a bithiazole structure. While thiazoline is the direct product of the Cy domain, the thiazoline-to-thiazole conversion generally is performed with an additional oxidation step. We identified at the C-terminus of NRPS-0 an additional domain that shows low, but significant, sequence homology to a family of putative oxidases/dehydrogenases, including the McbC protein of the microcin B17 synthase (Table 1). Microcin B17 synthase catalyzes the synthesis of the oxazole and thiazole-containing peptide antibiotic microcin B17, and McbC has been proposed to play a role in catalyzing the oxazoline/thiazoline-to-oxazole/thiazole conversion. Consequently, we propose that this extra domain at the C-terminus of NRPS-0 provides the oxidase/dehydrogenase activity for the biosynthesis of the bithiazole moiety of BLM, defining a novel Ox domain for NRPSs.

It is noteworthy that a cell-free preparation from *Sv* ATCC15003 has been reported to catalyze the conversion of phleomycins to BLMs in the presence of NAD^+, supporting the hypothesis that the bithiazole moiety of BLM results from stepwise oxidations of a bithiazoline precursor (Fig. 1A). (The phleomycin producer could be imagined to result from the loss of its Ox activity for the first thiazoline ring.) Given the wide distribution of thiazole or oxazole rings in natural products exhibiting an impressive array of biological activities, the cloning of the *blmIV, III* genes and the identification of the Ox domain open many opportunities thiazole biosynthesis and to synthesize novel thiazole containing molecules by engineering peptide biosynthesis.

Representative thiazole syntheses using variants of the blm NRPS are illustrated in Figure 6. Note that in Figure 6, A^M and A^N refer to an A domain that activates and amino acid with R^M and R^N groups, respectively. A^C refers to an A domain that activates Cys (x = SH) or Ser (X = OH) that can be cyclized to form the oxiaoline/thiazoline or oxazole/thiazole structures. DH is a dehydratase. In view of these representative examples, one of skill in the art would appreciate that the *blm NRPS* domain and its variants can be used in a wide variety of chemical syntheses make thiazolidine, thiazoline, thiazole, bi-thiazolidine, bithiazoline, or bithiazole-containing compounds.

3. Use of the *blm* gene cluster to make heterocyclic ring-containing compounds.

Various *blm* modules can be used to produce heterocyclic ring-containing compounds. Such heterocycles include, but are not limited to five member S- and N-containg compounds of the thiazolidine, thiazoline and thiazole family or the O- and N-containing compounds of the oxazolidine, oxazoline, and oxazole family. Again, the preparation of such compounds is illustrated in Figure 6.

4. Use of the *blm* gene cluster to make sugars.

In still another embodiment, the *blm* gene cluster or elements thereof can be used to make sugars. Such sugars include, but are not limited to L-sugars (with the *BlmG* epimerase), sugars modified by a carbamoyl group (*e.g.*, using *BlmD*), and various disaccharides. Representative examples of such syntheses are illustrated in Figure 7. Such sugar biosynthesis genes can also e used to attach sugars onto other polyketide and/or peptide aglycones.

F) Screening of products.

Particularly where large combinatorial libraries are synthesized, *e.g.* using one or more modules and/or enzymatic domains of the *blm* gene cluster it will often be desired to screen the resulting compound(s) for the desired activity. Mehtods of screening compounds (*e.g.* polypeptides, polyketides, sugars, thiazoles, *etc.*) for various activities of interest (*e.g.* cytotoxicity, antimicrobial activity, particular chemical activities, *etc.*) are well known to those of skill in the art.

Where large numbers of compounds are produced, it is often desired to rapidly screen such compounds using "high throughput systems" (HTS). High throughput assays systems are well known to those of skill in the art and many such systems are commercially available. (*see, e.g.*, Zymark Corp., Hopkinton, MA; Air Technical Industries, Mentor, OH; Beckman Instruments, Inc. Fullerton, CA; Precision Systems, Inc., Natick, MA, etc.). These systems typically automate entire procedures including all sample and reagent pipetting, liquid dispensing, timed incubations, and final readings of the microplate in detector(s) appropriate for the assay. These configurable systems provide high throughputand rapid start up as well as a high degree of flexibility and customization. The manufacturers of such systems typically provide detailed protocols for the various high throughput screens.

VII. *In Vitro* syntheses.

In additional embodiments of this invention, bleomycins and other polyketides and/or polypeptides are synthesized and/or modified *in vitro*. Individual enzymatic domains or modules can be used *in vitro* to modify a unit and/or to add a single monomeric unit to a growing polyketide or polypeptide chain. In one approach a metasynthetase providing all the desired synthetic activities recombinantly expressed and then provided, the appropriate substrates and buffer system *e.g.* in a bioreactor, to direct the synthesis of the desired product. In another approach, various PKSs and/or NRPSs are provided in different solutions and the growing polymer chains can be sequentially introduced into the plurality of solutions, each containing a single (or several) PKS or NRPS modules. In still another embodiment, the PKS and/or NRPS modules or enzymatic domains are provided attached to a solid support and a fluid contgaining the growing macromolecule is passed over the surface whereby the PKSs or NRPSs are able to react with the target substrate.

In one preferred embodiment, a combinatorial library of polyketides or polypeptides, or combinations thereof, is created by using automated means to facilitate the sequential introduction of a multitude of polymeric chains, each attached to a solid support, to a collection of solutions, each containing a single PKS or NRPS module. These automated means can be used to systematically vary the sequence by which each polymeric chain is introduced into the various solutions, thereby creating a combinatorial library. Numerous methods are well known in the art to create combinatorial libraries of molecules by the sequential addition of monomeric units, for example as described in WO 97/02358.

VIII. Kits.

In still another embodiment, this invention provides kits for practice of the methods described herein. In one preferred embodiment, the kits comprise one or more containers containing nucleic acids encoding one or more of the *blm* gene cluster ORFs and/or one or more of the BLM PKS or NRPS modules or enzymatic domains. Certain kits may comprise vectors encoding the *blm* orfs and/or cells containing such vectors. The kits may optionally include any reagents and/or apparatus to facilitate practice of the assays described herein. Such reagents include, but are not limited to buffers, labels, labeled antibodies, bioreactors, cells, *etc.*

In addition, the kits may include instructional materials containing directions (*i.e.*, protocols) for the practice of the methods of this invention. Preferred instructional

materials provide protocols utilizing the kit contents for creating or modifying *blm* module or ORF and/or for synthesizing or modifying a molecule using one or more *blm* modules and/or enzymatic domains. While the instructional materials typically comprise written or printed materials they are not limited to such. Any medium capable of storing such instructions and communicating them to an end user is contemplated by this invention. Such media include, but are not limited to electronic storage media (e.g., magnetic discs, tapes, cartridges, chips), optical media (e.g., CD ROM), and the like. Such media may include addresses to internet sites that provide such instructional materials.

EXAMPLES

The following examples are offered to illustrate, but not to limit the claimed invention.

Example 1

Bleomycin biosynthesis in *Streptomyces verticillus* ATCC15003, A model for hybrid peptide and polyketide biosynthesis.

Here we report the cloning and characterization of the *blm* biosynthesis gene cluster from *Sv* ATCC15003 (Fig. 2). Sequence analysis and biochemical characterization of individual modules enabled us to align the nine NRPS and one PKS modules in a linear order to constitute the Blm megasynthetase complex (Fig. 1B). These studies revealed several unprecedented features for peptide and polyketide biosynthesis, setting the stage to investigate the molecular basis for intermodular communication between NRPS and PKS, and supported the wisdom of combining individual NRPS and PKS modules for combinatorial biosynthesis to make novel "unnatural" natural products from amino acids and short carboxylic acids.

Materials and Methods.

General procedures.

Escherichia coli DH5α (Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual,* 2nd ed, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, USA), *E. coli* XL 1-Blue MR (Stratagene, La Jolla, CA), *E. coli* BL21(DE-3) (Novagen, Madison, WI), and *Sv* ATCC15003 (American Type Culture Collection, Rockville, MD) were used in this work. pOJ446 (Agricultural Research Service Culture Collection, Peoria, IL), pQE60 (Qiagen, Santa Clarita, CA), pET28a and pET29a (Novagen), and other plasmids

were from commercial sources. *E. coli* (Sambrook, *supra.*) and *Sv* ATCC15003 strains (Hopwood *et al.* (1985) *Genetic Manipulation of Streptomyces: A Laboratory Manual*, The John Innes Foundation, Norwich, UK) were cultured under standard conditions.

Plasmid preparation was carried out by using commercial kits (Qiagen). Total *Sv* ATCC15003 DNA was isolated according to literature protocols (Hopwood *et al.* (1985) *Genetic Manipulation of Streptomyces: A Laboratory Manual*, The John Innes Foundation, Norwich, UK; Nagaraja *et al.* (1987) *Methods Enzymol.* 153: 166-198). Restriction enzymes and other molecular biology reagents were from commercial sources, and digestions and ligation followed standard methods (Sambrook, *supra.*). For Southern analysis, digoxigenin labelling of DNA probes, hybridization, and detection were performed according to the protocols provided by the manufacturer (Boehringer Mannheim Biochemicals, Indianapolis, IN).

Automated DNA sequencing was carried out on an ABI Prism 377 DNA Sequencer (Perkin-Elmer/ABI, Foster City, CA), and this service was provided by either the DBS Automated DNA Sequencing Facility, UC Davis, or Davis Sequencing (Davis, CA). Data were analyzed by the ABI Prism Sequencing 2.1.1 software and the Genetics Computer Group (GCG) program (Madison, WI).

Cloning and sequencing of the *blm* gene cluster.

A genomic library of *Sv* ATCC15003 was constructed in pOJ446 according to literature procedures (Nagaraja *et al.* (1987) *Methods Enzymol.* 153: 166-198) and screened with probes made from both ends of the *blmAB* locus (Sugiyama *et al.* (1994) *Gene* 151: 11-16; Calcutt and Schmidt (1994) *Gene* 151: 17-21), leading to the localization of 140-kb contiguous DNA, of which 100-kb is upstream (Fig. 2) and 40-kb is downstream (data not shown) of the *blmAB* genes. Heterologous NRPS probes were amplified from *Sv* ATCC15003 by polymerase chain reaction (PCR) according to literature procedures (Turgay and Marahiel (1994) *Peptide Res.* 7: 238-241) and used to screen the entire 140-kb DNA by Southern analysis under various hybridization conditions (Shen *et al.* (1999) *Bioorg. Chem.* 27: 155-171).

Prediction of substrate specificity of NRPSs.

The nine Blm NRPS modules were compared with eighty four modules from various bacterial and fungal NRPSs available at the GenBank, including those with known or putative specificity for amino acids present in BLM. A table of overall similarities/identities

was generated by PILEUP analysis of the A3 to A6 regions, and the residues lining the substrate binding pocket by comparison with PheA (Conti *et al.* (1997) *EMBO J.* 16, 4174-4183) were determined by PILEUP/PRETTY analysis. The percentage similarities for each Blm NRPS module were plotted against the rest of the NRPS modules to display the overall sequence homology between the A3 to A6 region. Those modules that showed significantly higher homology were selected to compare the amino acid residues that line the substrate binding pocket.

Overproduction and biochemical characterization of the NRPS-1A and NRPS-6A proteins.

Heterologous expression of the A domain in *E. coli* were performed according to literature procedures (Mootz and Marahiel (1997) *J. Bacteriol.* 179: 6843-6850). NRPS-1A (forward primer 5'-AAC CCA TGG CTG CTT CCC TGA CCC GCC TGG CC-3', SEQ ID NO:76, and reverse primer 5'-CCT AGA TCT ACG GGC AGG TGG GGC GGT-3', SEQ ID NO:77) and NRPS-6A (forward primer 5'-GGG AAT TCC ATA TGA TCC TCA CGT CCT TCC AC-3', SEQ ID NO:78, and reverse primer 5'-GGC AAG CTT GGG TGA GGG TCC GTT CGG T-3', SEQ ID NO:79) were amplified by PCR from *Sv* ATCC15003 cosmid clones. The resulting 1.6-kb fragment of NRPS-1A was first cloned into the *NcoI/BglII* sites of pQE60 and then moved as an *NcoI/HindIII* fragment into the similar sites of pET29a to yield pBS10, and the resulting 1.6-kb fragment of NRPS-6A was directly cloned into the *NdeI/HindIII* sites of pET28a to yield pBS11. Introduction of pBS10 and pBS11 into *E. coli* BL21(DE-3) under standard expression conditions resulted in production of NRPS-1A (with an N-terminal S-tag and a C-terminal His_6-tag) and NRPS-6A (with an N-terminal His_6-tag), respectively. The soluble fractions of fusion proteins were subjected sequentially to an affinity chromatography on Ni-NTA resin and an anion exchange chromatography on a Hyper-D column (PerSeptive Biosystem, Framingham, MA), resulting in NRPS-1A and NRPS-6A with near homogeneity.

Results and Discussion.

Cloning of the *blm* gene cluster from *Sv* ATCC15003.

Davies and co-workers previously cloned two BLM resistance genes (*blmA* and *blmB*) from *Sv* ATCC15003 (Sugiyama *et al.* (1994) *Gene* 151: 11-16), and Calcutt and Schmidt (1994) *Gene*, 151: 17-21, sequenced a 7.2-kb DNA fragment flanking the *blmAB*

genes, revealing seven open reading frames (orfs), none of which were found to encode Blm NRPS or PKS enzymes. Given the precedent that antibiotic production genes commonly occur as a cluster in actinomycetes, we adopted an approach combining chromosomal walking from the *blmAB* resistance locus and DNA hybridization with heterologous NRPS probes to clone and identify the *blm* cluster, leading to the localization of 140-kb contiguous *Sv* ATCC15003 DNA. DNA sequencing of approximately 90-kb of the *blm* gene cluster, including the 7.2-kb *blmAB* locus, revealed 40 ORFs (Fig. 2). Preliminary functional assignments were made by comparison of the deduced gene products with proteins of known functions in the database. Among the ORFs identified from the *blm* cluster, we indeed found a PKS module, flanked by several NRPS modules–a fact that supports the hybrid NRPS/PKS/NRPS hypothesis for BLM biosynthesis–along with several sugar biosynthesis genes and genes encoding other biosynthesis enzymes as well as several resistance and regulatory genes (Table 1).

Noteworthy are the genes encoding the putative NRPS and PKS enzymes. The *blmI, blmII,* and *blmXI* genes encode NRPSs with an unusual architecture. In contrast to all known NRPSs, which are of modular organization with each module consisting minimally of a condensation (C), an adenylation (A), and a peptidyl carrier protein (PCP) domain (1), BlmI, BlmII, and BlmXI are discrete proteins homologous to individual domains of type I NRPSs. We have characterized BlmI as a type II PCP (18). The BlmII and BlmXI proteins could serve as candidates for type II condensation enzymes. It is unclear yet what role if any these discrete NRPS enzymes could play in BLM biosynthesis.

The *blmIII, blmIV, blmV, blmVI, blmVII, blmIX,* and *blmX* genes encode modular NRPSs consisting of domains characteristic for known type I NRPSs (A special thematic issue on polyketide and nonribosomal polypeptide biosynthesis, (1997) *Chem. Rev.* 97: 2463-2706), such as the A, PCP, C, and condensation/cyclization (Cy) domains (Konz *et al.* (1997) *Chem. Biol.* 4: 927-937), as well as an unprecedented oxidation (Ox) domain (see discussion below). However, BlmVI is unique among all the Blm NRPSs identified. Its N-terminal module (NRPS-5) consists of an atypical A domain, which bears a close resemblance to a family of acyl CoA synthases (Fitzmaurice and Kolattukudy (1997) *J. Bacteriol.* 179: 2608-2615; Fitzmaurice and Kolattukudy (1998) *J. Biol. Chem.* 273: 8033-8039), and an acyl carrier protein (ACP)-like domain (A special thematic issue on polyketide and nonribosomal polypeptide biosynthesis, (1997) *Chem. Rev.* 97: 2463-2706). Its C-terminal module is truncated and presumably interacts with BlmV to constitute the complete NRPS-3 module (Fig. 1B). Also noteworthy are the C domain of NRPS-3 that lacks both

His residues of the conserved HHxxxDG (SEQ ID NO:4) active site for transpeptidation (Stachelhaus *et al.* (1998) *J. Biol. Chem.*, 273: 22773-22781) and the extra C domain at the C-terminus of BlmV. These unusual features associated with *BlmVI* and *BlmV* may play roles in the formation of the β-aminoalaninamide and the pyrimidine moieties of BLM, which are unprecedented in peptide biosynthesis. For example, we propose that the NRPS-4-activated Ser is first dehydrated into dehydroalanine before condensation–an analogous Thr-to-2,3-dehydroaminobutyric acid dehydration has been observed in syringomycin biosynthesis (Guenzi *et al.* (1998) *J. Biol. Chem.* 273: 32857-32863). Conjugate addition to dehydroalanine by Asn on the NRPS-3 module downstream followed by an aminolysis to cleave the Ser-Asn adduct off the Blm megasynthetase furnishes the β-aminoalaninamide moiety (Fig. 1B). The former reaction could be catalyzed by the C domain of NRPS-3 that apparently is nonfunctional for normal transpeptidation due to the lack of the active sites, and the latter reaction could be catalyzed by the acyl CoA synthase-like domain of NRPS-5 in a process that resembles the acyl CoA synthase-catalyzed synthesis of acyl CoA from carboxylic acid (Stachelhaus *et al.* (1998) *J. Biol. Chem.* 273: 22773-22781; Guenzi *et al.* (1998) *J. Biol. Chem.* 273: 32857-32863) but in the reverse direction in the presence of an amino donor (Fig. 1B).

The *blmVIII* gene encodes a PKS module consisting of domains characteristic for known PKSs, such as ketoacyl synthase (KS), acyltransferase (AT), ketoreductase (KR), and ACP, with malonyl CoA acting as an extending unit according to sequence comparison of the AT domain (Haydock *et al.* (1995) *FEBS Lett.* 374: 246-248) (Fig. 1B). However, the identification of an integrated methyltransferase (MT) domain (Kagan and Clarke (1994) *Arch. Biochem. Biophys.* 310: 417-427) in the middle of *BlmVIII* is unique, representing the first PKS from actinomycetes that contains an internal MT domain. The only other example of PKS from bacteria that contains an internal MT domain is HMWP1 of the yersiniabactin gene cluster (Pelludat *et al.* (1998) *J. Bacteriol.* 180: 538-546). It has been assumed that fungal PKSs in general contain internal MTs for the introduction of methyl branch into the polyketide products, as it has been shown recently in lovastatin biosynthesis (Kennedy *et al.* (1999) *Science* 284: 1368-1372).

The Blm megasynthetase-templated assembly of BLM.

According to the hybrid NRPS/PKS/NRPS model for BLM biosynthesis (Fig. 1A), we predict a linear modular organization of individual NRPS and PKS modules to constitute the Blm megasynthetase. Thus, the first functional domain of the Blm

megasynthetase should be a NRPS module that initiates BLM biosynthesis by activating L-Ser as an amino acylthioester to set the stage for transpeptidation. Chain elongation proceeds by sequential incorporation of L-Asn, L-Asn, L-His, and L-Ala, requiring four additional NRPS modules. In the next step, a malonate reacts with the resulting pentapeptide intermediate to form a β-ketothioester intermediate that is subsequently methylated at the α-position and reduced at the β-keto group. A PKS module presumably dictates all these biosynthetic events and interacts with the aligned NRPS module upstream to channel the growing peptide intermediate from an NRPS module to a PKS module. After one cycle of polyketide elongation, peptide elongation is resumed by incorporation of an L-Thr residue. This step is presumably catalyzed by an NRPS module that interacts with the upstream PKS module to channel the growing polyketide intermediate (as far as the active site is concerned) from a PKS module to an NRPS module. At this stage, methylation occurs at the pyrimidine moiety of the growing intermediate, presumably catalyzed by a discrete methyltransferase; chain elongation is continued by three additional NRPS modules that incorporate a β-Ala and two L-Cys molecules sequentially. Finally, the fully assembled BLM peptide/polyketide/peptide backbone is hydroxylated at the β-position of the His residue, presumably by a discrete hydroxylase, and released from the Blm megasynthetase complex via nucleophilic substitution of the RCO-S-PCP species by a terminal amine to form the BLM aglycone. Intermediates after five of the nine proposed elongation steps were in fact isolated as P-3, P-3A, P-3K, P-4, P-5, P-5m, P-6m, and P-6mo (Takita and Muroka (1990) pages 289-309 in *Biochemistry of Peptide Antibiotics: Recent Advances in the Biotechnology of β-Lactams and Microbial Peptides*, Kleinkauf, H. & von Döhren, H. eds., W. de Gruyter, N.Y.), which presumably resulted from premature departure from the Blm megasynthetase complex before the chain reaches its full length (Fig. 1B).

Most of the bacterial NRPS gene clusters characterized to date are organized in operon-type structures, encoding multimodular NRPS proteins with individual modules organized along the chromosome in a linear order that parallels the order of the amino acids in the resultant peptides, i.e., following the "colinearity rule" for the NRPS-templated assembly of peptides from amino acids (A special thematic issue on polyketide and nonribosomal polypeptide biosynthesis, (1997) *Chem. Rev.* 97: 2463-2706; Cane *et al.* (1998) *Science* 282: 63-68). Inspection of the *blm* gene cluster (Fig. 2) showed that the Blm NRPS and PKS modules apparently are not organized according to the "colinearity rule" for BLM biosynthesis (Fig. 1). [Exception to the "colinearity rule" was also noted in the

syringomycin synthetase gene cluster (Guenzi *et al.* (1998) *J. Biol. Chem.* 273: 32857-32863), and in fact, Grandi and co-workers have demonstrated recently in *Bacillus subtilis* that neither the operon-type structure nor the physical linkage of individual modules is essential for proper assembly and activity of the surfactin NRPS megasynthetase (Guenzi *et al.* (1998) *J. Biol. Chem.* 273: 14403-14410).] Realizing that the BLM biosynthesis cannot be rationalized according to the "colinearity rule", we determined the substrate specificity of individual NRPS and PKS modules in an attempt to shed light on the modular organization of the Blm megasynthetase complex. Brick and co-workers postulated, based on the X-ray structural analysis of the A domain of GrsA, PheA, that the region between core sequences A3 to A6 represent the amino acid specificity determinant of an NRPS module (Conti *et al.* (1997) *EMBO J.* 16: 4174-4183). Since the A domains in all known NRPSs share a significant sequence identity (ensuring that the main chain conformation of the enzymes is likely to be very similar), they further proposed that the differing substrate specificity of individual NRPS modules will be mainly determined by the nature of the amino acids lining the substrate binding pocket (Stachelhaus *et al.* (1999) *Chem. Biol.* 6: 493-505; Conti *et al.* (1997) *EMBO J.* 16: 4174-4183). Given this structural information and the vast amount of NRPS sequences available at the GenBank, we developed a novel approach for predicting substrate specificity for NRPS modules by comparing the overall sequence between the A3 to A6 region and the eight amino acid residues that line up the substrate binding pocket. While a constant level of similarities (30%-40%) was evident among all the NRPS modules analyzed, most of the Blm NRPS modules showed striking similarities (50%-60%) to a particular cluster of NRPS modules as exemplified in Fig. 3A for NRPS-1 and NRPS-6. Close examination of these modules clustered with higher similarities revealed that they activate the same or very similar amino acid, based on which the putative substrate for the NRPS in query could be predicted, i.e., NRPS-1 and NRPS-6A activate L-Cys and L-Thr, respectively. These predictions were further supported by comparing the residues lining the substrate binding pocket. For example, the amino acid residues lining the substrate binding pocket for NRPS-1 and NRPS-6 are almost identical to those NRPS modules that are known to activate L-Cys and L-Thr, respectively, as shown in Fig. 3B. To verify the predicted amino acid specificity, we overproduced and purified the NRPS-1A and NRPS-6A proteins (Fig. 3C) and examined their substrate specificity according to the amino acid-dependent ATP-PPi assay (Lee *et al.* (1970 *Meth. Enzymol.*, 43: 585-602; Ku *et al.* (1997) *Chem. & Biol.*, 4: 203-207). NRPS-1A and NRPS-6A indeed activate specifically L-Cys and L-Thr, respectively, among the amino acids tested (Fig. 3D). The latter results greatly enhanced our

confidence in predicting the substrate specificity of a NRPS module by the above method. We subsequently determined the substrate specificity for all the NRPS modules identified from the *blm* gene cluster and they in fact accounted for all nine amino acids required for BLM biosynthesis (Fig. 2).

Using the substrate specificity of individual NRPS and PKS modules as a guide, we can align the nine NRPS and one PKS modules to constitute the Blm megasynthetase as shown in Fig. 1B according to our hybrid NRPS/PKS/NRPS model for BLM biosynthesis (Fig. 1A). Among all the PKSs or NRPS systems examined so far, the Blm megasynthetase consists of the largest number of individual proteins. The precise interactions among all the Blm NRPS and Blm PKS proteins to constitute the Blm megasynthetase complex, therefore, reflect a remarkable power of protein-protein recognition (Guenzi *et al.* (1998) *J. Biol. Chem.* 273: 14403-14410; Gokhale *et al.* (1999) *Science* 284: 482-485). Although we are yet to provide direct evidence supporting the specific protein-protein interactions between the neighboring proteins, it is striking to note that all the biosynthetic intermediates isolated are derailed from either PKS or NRPS modules at the junctions between the interacting proteins (Fig. 1B). Since it is not difficult to imagine that an intermediate is more likely to fall off the enzyme complex when it is subjected to interpeptide transfer than to intrapeptide transfer, we view the latter observation as strong evidence supporting the current model of the Blm megasynthetase

BlmIX/BlmVIII/BlmVII as a hybrid NRPS/PKS/NRPS model.

Recent biosynthetic studies on rapamycin in *Streptomyces hygroscopicus* (Konig *et al.* (1997) *Eur. J. Biochem.* 247: 526-534), yersiniabactin in *Yersinia enterocolitica* and *Y. pestis* (Pelludat *et al.* (1998) *J. Bacteriol.* 180: 538-546; Gehring *et al.* (1998) *Chem. Biol.* 5: 573-586; Gehring *et al.* (1998) *Biochemistry* 37: 11637-11650) and TA in *Myxococcus xanthus* (Paitan *et al.* (1999) *J. Mol. Biol.* 286, 465-474) are starting to shed light on hybrid peptide and polyketide biosynthesis. Two models are emerging for the alignment between a NRPS and a PKS module. The interacting NRPS and PKS modules could be either covalently linked by arranging all domains in a linear order on the same protein (Pelludat *et al.* (1998) *J. Bacteriol.* 180: 538-546; Gehring *et al.* (1998) *Chem. Biol.* 5: 573-586; Gehring *et al.* (1998) *Biochemistry* 37: 11637-11650; Paitan *et al.* (1999) *J. Mol. Biol.* 286: 465-474) or physically located on two separate proteins, requiring specific protein-protein recognition to ensure the correct pairing between the interacting modules (Pelludat *et al.* (1998) *J. Bacteriol.* 180: 538-546; Konig *et al.* (1997) *Eur. J. Biochem.* 247: 526-534;

Gehring *et al.* (1998) *Chem. Biol.* 5: 573-586; Gehring *et al.* (1998) *Biochemistry* 37: 11637-11650). Common to all these systems, however, are the unusual features associated with the interacting modules, such as the lack of the AT domain of the PKS module in Tal (Paitan *et al.* (1999) *J. Mol. Biol.* 286: 465-474) and the lack of the A domain and the presence of the Cy domain of the NRPS modules in both HMWP1 and HMWP2 (Pelludat *et al.* (1998) *J. Bacteriol.* 180: 538-5461; Gehring *et al.* (1998) *Chem. Biol.* 5: 573-586; Gehring *et al.* (1998) *Biochemistry* 37: 11637-11650). While extremely intriguing, the latter features complicate mechanistic analysis of these systems, making them less ideal candidates for studying how NRPS and PKS integrate into a productive hybrid NRPS/PKS complex.

The *BlmIX/BlmVIII/BlmVII* system combines the features of both hybrid NRPS/PKS and PKS/NRPS systems, serving as an ideal model for studying hybrid peptide and polyketide biosynthesis. The fact that both the *BlmIX* and *BlmVII NRPS* modules and the *BlmVIII* PKS module themselves are three separate proteins with a typical domain organization for NRPS and PKS enzymes greatly simplifies the mechanistic analysis of the hybrid NRPS/PKS/NRPS complex. We have found that the KS domain of BlmVIII is more similar to the KSs of HMWP1 (Pelludat *et al.* (1998) *J. Bacteriol.* 180: 538-546) and Tal (Paitan *et al.* (1999) *J. Mol. Biol.* 286: 465-474), both of which catalyze the elongation of a peptidyl intermediate with a malonate, than to KSs of type I PKSs. We attribute these subtle differences to their unique reactivity that catalyzes the transfer of the peptidyl intermediate from the PCP to the KS domain, which presumably takes place prior to chain elongation (Fig.4). Subsequent condensation catalyzed by the KS domain between the peptidyl intermediate and malonyl-S-ACP results in the elongation of the growing peptide with a carboxylic acid. Equally striking are the discoveries that the ACP domain of BlmVIII is more similar to a PCP than to an ACP and that the C domain of BlmVII has an additional N-terminal segment of about 50 amino acids that is rich in arginine, aspartic acid, and glutamic acid. The latter feature is analogous to the N-terminal interpolypeptide linker for type I PKS, which has recently been demonstrated to play a critical role in intermodular communication (Gokhale *et al.* (1999) *Science* 284: 482-485). We propose that these unique features of the ACP domain from the BlmVIII PKS module and the C domain from the BlmVII NRPS module provide the molecular basis for the C domain to recognize the acyl-S-ACP as a substrate. Subsequent condensation catalyzed by the C domain between acyl-S-ACP and amino acyl-S-PCP results in the elongation of the growing polyketide (as far as this condensation is concerned) with an amino acid (Fig. 4).

Novel domains for the Blm NRPS and PKS modules.

Various NRPS and PKS domains have been characterized, which are the building blocks for the entire field of combinatorial biosynthesis. The success for combinatorial biosynthesis depends critically upon the repertoire of these individual domains. Genetic analysis of the *blm* gene cluster has uncovered several novel NRPS and PKS domains. Without being bound to a particular theory, it is believed that *BlmVI* and *BlmV* are involved in the biosynthesis of the β-aminoalaninamide and pyrimidine moieties of BLM). In addition, the MT domain in BlmVIII, the Cy domains in BlmIV, and the Ox domain in BlmIII are novel domains.

The BlmVIII PKS module apparently furnishes the "propionate" unit into BLM in two steps by evolving a malonyl CoA-specifying AT domain coupled with a novel S-adenosylmethionine-requiring MT domain, representing a new mechanism to introduce methyl branches into polyketides (Fig. 4). This biosynthetic reaction sequence is unprecedented for polyketide biosynthesis since all PKSs from actinomycetes examined to date incorporate the alkyl branches into the resultant polyketides by selecting various alkyl malonates as the extending units that are determined by the AT domains. Yet, feeding experiments have unambiguously established that the polyketide moiety of BLM was derived from an acetate and a methionine (Takita and Muroka (1990) pages 289-309 in *Biochemistry of Peptide Antibiotics: Recent Advances in the Biotechnology of β-Lactams and Microbial Peptides*, Kleinkauf, H. & von Döhren, H. eds., W. de Gruyter, N.Y.), a fact that fits well with the observed unusual domain organization of the BlmVIII PKS module (Fig. 4). It is conceivable that the combination of this MT domain with an AT domain specific for a methyl malonate extending unit (Haydock *et al.* (1995) *FEBS Lett.* 374: 246-248) could result in the synthesis of polyketides with a gem-dimethyl moiety via engineering polyketide biosynthesis. Such a gem-dimethyl group has been found to be a very important pharmacophore for the epothilones, a family of hybrid peptide and polyketide metabolites that exhibits a remarkable antitumor activity similar to taxol (Ojima *et alo.* (1999) *Proc. Natl. Acad. Sci. USA* 96: 4256-4261).

The BlmIV and BlmIII NRPSs are characterized by the unusual Cy domains as well as the unprecedented Ox domain, providing an efficient biosynthesis for a bithiazole structure. The Cy domain was first defined by Marahiel and co-workers in their study of bacitracin biosynthesis in *B. licheniformis* (Konz *et al.* (1997) *Chem. Biol.* 4: 927-937), and the Cy activity was demonstrated recently by Walsh and co-workers in their study of the

HMWP1 and HMWP2 proteins for yersiniabactin biosynthesis in *Y. pestis* (Gehring *et al.* (1998) *Chem. Biol.* 5: 573-586; Gehring *et al.* (1998) *Biochemistry* 37: 11637-11650). While thiazoline is the direct product of the Cy domain, the thiazoline-to-thiazole conversion requires an additional oxidation step. We identified at the C-terminus of NRPS-0 an additional domain that shows low, but significant, sequence homology to a family of putative oxidases/dehydrogenases, including the McbC protein of the microcin B17 synthase (Table 1). Microcin B17 synthase catalyzes the synthesis of the oxazole and thiazole-containing peptide antibiotic microcin B17, and McbC has been proposed to play a role in catalyzing the oxazoline/thiazoline-to-oxazole/thiazole conversion (Li *et al.* (1996) *Science* 274: 1188-1193; Milne, *et al.* (1999) *Biochemistry* 38: 4768-4781). Consequently, we propose that this extra domain at the C-terminus of NRPS-0 could provide the oxidase/dehydrogenase activity needed for the biosynthesis of the bithiazole moiety of BLM, defining a novel Ox domain for NRPSs. It is noteworthy that a cell-free preparation from *Sv* ATCC15003 has been reported to catalyze the conversion of phleomycins to BLMs in the presence of NAD^+ (Takita and Muroka (1990) pages 289-309 in *Biochemistry of Peptide Antibiotics: Recent Advances in the Biotechnology of β-Lactams and Microbial Peptides*, Kleinkauf, H. & von Döhren, H. eds., W. de Gruyter, N.Y.), supporting the hypothesis that the bithiazole moiety of BLM results from stepwise oxidations of a bithiazoline precursor (Fig. 1A). (The phleomycin producer could be imagined to result from the loss of its Ox activity for the first thiazoline ring.) Given the wide distribution of thiazole or oxazole rings in natural products (Ojima *et alo.* (1999) *Proc. Natl. Acad. Sci. USA* 96: 4256-4261; Li *et al.* (1996) *Science* 274: 1188-1193) exhibiting an impressive array of biological activities, the cloning of the *blmIV,III* genes and the identification of the Ox domain open many opportunities to define the mechanism for thiazole biosynthesis and to potentially synthesize novel thiazole containing molecules by engineering peptide biosynthesis.

Example 2

Identification and characterization of a type II peptidyl carrier protein from the bleomycin producer *Streptomyces verticillus* ATCC 15003.

Results.

Cloning and sequence analysis of the *blmI* gene

In our effort to clone the gene cluster responsible for BLM biosynthesis, we have determined 80 kb DNA sequence from *Sv* ATCC15003 (Fig. 8). Among the orfs identified within the *blm* gene cluster is the small orf of 273 base pairs (bp), *blmI*, which is located approximately 4 kb upstream of the previously characterized *blmAB* resistance locus (Sugiyama *et al.* (1994) *Gene* 151: 11-16; Calcutt and Schmidt (1994) *Gene* 151: 17-21) (Fig. 8B). The *blmI* gene encodes a protein of 90 amino acids with a molecular weight of 9957 and a pI of 6.52 (Fig. 8C). Computer-assisted analysis (Altschul *et al.* (1997) *Nucleic Acids Res.* 25: 3389-3402) of the deduced amino acid sequence indicates that BlmI is very similar to various PCP domains of NRPSs (ranging around 40% identity and 60% similarity, as shown in Figure 9). Like known PCP domains of NRPS, BlmI has the highly conserved signature motif of LGGXS, within which the serine residue is the site for 4'-phosphopantetheinylation (Stachelhaus and Marahiel (1995) *FEMS Microbiol. Lett.* 125: 3-14; Marahiel *et al.* (1997) *Chem. Rev.* 97: 2651-2673). The latter posttranslational modification is generally necessary for peptide biosynthesis; converting the apo-PCP into the functional holo-PCP (Marahiel *et al.* (1997) *Chem. Rev.* 97: 2651-2673; Walsh *et al.* (1997) *Curr. Opin. Chem. Biol.* 1: 309-315). Based on sequence comparison, *BlmI* is most related to PCPs and not to other kinds of carrier proteins that also share the same LGGXS (SEQ ID NO:80) motif and undergo the same posttranslational 4'-phosphopantetheinylation [31], such as the *E. coli* acyl carrier protein (ACP) (Lambalot and Walsh (1995) *J. Biol. Chem.* 270: 24658-24661), the ACP domain of type I PKS and the type II PKS ACP (Cox and Simpson (1997) *FEBS Lett.* 405: 267-272; Carreras *et al.* (1997) *Biochemistry* 36: 11757-11761), the ArCP domain (Gehring *et al.* (1998) *Biochemistry* 37: 2648-2659), and several nodulation related ACP-like proteins (Epple *et al.* (1998) *J. Bacteriol.* 180: 4950-4954; Spaink *et al.* (1991) *Nature* 354: 125-130).

Overexpression of *blmI* in *E. coli*

To overexpress the *blmI* gene in *E. coli*, we directly amplified the *blmI* gene by PCR from the *Sv.* ATCC15003 genomic DNA and cloned it into the pQE-60 expression vector to give pBS1 so that BlmI could be produced as a protein with a native N-terminus and a His_6-tag at its C-terminus. However, no production of the BlmI protein was detected, as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), upon introduction of pBS1 into *E. coli* M15(pREP4) under the standard overexpression conditions recommended by the manufacturer (Qiagen). We reasoned that the small BlmI protein with its native N-terminus may not be stable in the heterologous host, and hence moved the *blmI* gene from pBS1 into pET-29a to yield the second overexpression construct of pBS2. In the latter construct, BlmI should be produced as a fusion protein with 27 extra amino acid residues at its N-terminus, including an S-tag and the thrombin cleaving site, in addition to the His_6-tag at its C-terminus. Introduction of pBS2 into *E. coli* BL21(DE-3) under the standard overexpression conditions recommended by the manufacturer (Novagen) indeed resulted in overproduction of BlmI. In fact, the bulk of the soluble protein was the overproduced BlmI, which was easily purified by affinity chromatography using Ni-NTA resin (Qiagen). It is noteworthy that fusion of the additional 23 amino acids to the N-terminus of BlmI as in pBS2 and change of the expression system from *E. coli* M15(pREP4) (pBS1) to *E. coli* BL21(DE-3)(pBS2) dramatically improved the expression level of *blmI*.

In vivo 4'-phosphopantetheinylation of the BlmI protein

To establish BlmI as a type II PCP, we tested if it could serve as a substrate for a PCP-specific 4'- PPTase. PPTases catalyze the posttranslational modification of an apo-PCP into a holo-PCP by transferring the 4'-phosphopantetheine moiety from co-enzyme A (CoA) to the conserved serine residue of PCP, and this reaction has been developed recently into a general method to prepare various holo-PCP, holo-ACP, or holo-ArCP from the corresponding apoproteins (Stachelhaus *et al.* (1996) *Chem. Biol.* 3: 913-9211; Gehring *et al.* (1998) *Biochemistry* 37: 2648-2659; Gehring *et al.* (1998) *Biochemistry* 37: 11637-11650; Weinreb *et al.* (1998) *Biochemistry* 37: 1575-1584). Therefore, we decided to investigate the 4'-phosphopantetheinylation of BlmI under both *in vivo* (Ku *et al.* (1997) *Chem. Biol.* 4: 203-207) and *in vitro* (Gehring *et al.* (1998) *Biochemistry* 37: 11637-11650; Lambalot *et al.* (1996) *Chem. Biol.* 3: 923-936; Quadri *et al.* (1998) *Biochemistry* 37: 1585-1595) conditions.

To examine 4'-phosphopantetheinylation of BlmI in vivo, we chose *E. coli* OG7001 as the expression host, which is a β-alanine auxotroph derived from *E. coli* BL21(DE3) by P1 co-transduction of the *panD* mutation from *E. coli* SJ16 (Epple *et al.* (1998) *J. Bacteriol.* 180: 4950-4954). Upon introduction of pBS2 into *E. coli* OG7001, *blmI* was exceptionally well expressed and the overproduced BlmI protein was readily purified. However, high performance liquid chromatography (HPLC) analysis showed that the purified BlmI was essentially in the apo-form (Fig. 10A), indicative that apo-BlmI was a poor substrate for the *E. coli* endogenous PPTases, such as EntD and ACP synthase (Lambalot *et al.* (1996) *Chem. Biol.* 3: 923-936; Walsh *et al.* (1997) *Curr. Opin. Chem. Biol.* 1: 309-315; Lambalot and Walsh (1995) *J. Biol. Chem.* 270: 24658-24661). To circumvent the poor endogenous PPTase activity, we next co-expressed *blmI* with the *gsp* gene, which was isolated from the gramicidin S producer *Bacillus brevis*, and encoded a PPTase that was known to 4'-phosphopantetheinylate heterologously produced PCPs in *E. coli* (Lambalot *et al.* (1996) *Chem. Biol.* 3: 923-936; Ku *et al.* (1997) *Chem. Biol.* 4: 203-207). We co-transformed pDPT-Gsp, in which the expression of the *gsp* gene was under the control of the T5/Lac promoter (Ku *et al.* (1997) *Chem. Biol.* 4: 203-207), and pBS2 into *E. coli* OG7001. *BlmI* was again very well expressed and the resulting BlmI protein was similarly purified. HPLC analysis showed that at least 60% of overproduced BlmI was modified into the holo-BlmI protein (Fig. 10B). (A PCP domain was similarly 4'-phosphopantetheinylated in vivo before by co-expressing *gsp* in *E. coli* using pDPT-Gsp, and approximately 80% of the PCP was produced in the holo-form (Ku *et al.* (1997) *Chem. Biol.* 4: 203-207).

We next cultured *E. coli* OG7001(pBS2) and *E. coli* OG7001(pBS2/pDPT-Gsp) in the presence of [3-^{3}H]-β-alanine, a known biosynthetic precursor of 4'-phosphopantetheine (Stachelhaus *et al.* (1996) *Chem. Biol.* 3: 913-921; Epple *et al.* (1998) *J. Bacteriol.* 180: 4950-4954). Specific incorporation of [3-^{3}H]-β-alanine into the 4'-phosphopantetheine moiety of holo-BlmI was determined by autoradiographic analysis. Thus, while fermentation of *E. coli* OG7001(pBS2) in the presence of [3-^{3}H]-β-alanine led to an IPTG-dependent overproduction of BlmI, little of the resulting BlmI protein was ^{3}H-labeled, indicative of being produced in the apo-form. In contrast, fermentation of *E. coli* OG7001(pBS2/pDPT-Gsp) in the presence of [3-^{3}H]-β-alanine resulted in a significant increase of IPTG-dependent incorporation of the ^{3}H-label into the overproduced BlmI protein, suggesting a specific incorporation of [3-^{3}H]-β-alanine into holo-BlmI, presumably in the 4'-phosphopanthetheine moiety. There were several additional proteins that were also

weakly labeled by [3-^{3}H]-β-alanine. However, both their expression and their incorporation by ^{3}H-label were independent from either IPTG induction or the presence of Gsp, hence these proteins were unrelated to BlmI. (Similar background labeling was reported before for in vivo 4'-phosphopantheteinylation of other PCP (Epple *et al.* (1998) *J. Bacteriol.* 180: 4950-4954)). We also purified the BlmI protein from *E. coli* OG7001(pBS2/pDPT-Gsp) and demonstrated that it was the holo-BlmI protein that was specifically associated with the ^{3}H-activity. Finally, we confirmed the identity of holo-BlmI by subjecting the purified BlmI protein to MALDI-Tof mass spectral analysis (Weinreb *et al.* (1998) *Biochemistry* 37: 1575-1584). BlmI produced in the absence of the Gsp PPTase yielded a single peak with a molecular weight of 13,952, suggesting that the produced BlmI protein is in the apo-form (calc., 13,949). In contrast, BlmI produced in the presence of Gsp yielded two species with molecular weight of 13,969 and 14,303, respectively. While the species with the molecular weight of 13,969 represents apo-BlmI, a molecular weight of 14,303 unambiguously confirmed the other protein as holo-BlmI (calc., 14,289). The latter result indicated that the purified BlmI consisted of both the apo- and holo-BlmI proteins, in agreement with the HPLC analysis results (Fig. 10B).

In vitro 4'-phosphopantetheinylation of the BlmI protein

To investigate 4'-phosphopantetheinylation of BlmI in vitro, we chose the Sfp protein as the preferred PPTase, which had been isolated before from the surfactin producer *Bacillus subtilis* (Nakano *et al.* (1992) *Mol. Gen. Genet.* 232: 313-321). (Overexpression of *gsp* in *E. coli* using pDPT-Gsp resulted in predominantly an insoluble Gsp protein (Ku *et al.* (1997) *Chem. Biol.* 4: 203-207). The Sfp PPTase was overproduced in *E. coli* MV1190(pUC8-Sfp) and purified to near homogeneity as described before (Quadri *et al.* (1998) *Biochem.*, 37: 1585-1595; Nakano *et al.* (1992) *Mol. Gen. Genet.*, 232: 313-321). Upon incubation of the purified apo-BlmI with [^{3}H-pantetheine]-CoA in the presence of the Sfp PPTase, we examined the covalent incorporation of the [^{3}H-pantetheine]-4'-phosphopantetheine moiety from CoA into holo-BlmI by autoradiographic analysis. Indeed, the apo-BlmI was quantitatively labeled by [^{3}H-pantetheine]-CoA, and no labeling was observed in the absence of either the apo-BlmI or the Sfp PPTase protein, demonstrating that the Sfp PPTase can recognize apo-BlmI as a substrate and specifically transfer the 4'-phosphopantetheine group from CoA into holo-BlmI.

In vitro aminoacylation of BlmI

Once we established BlmI as a type II PCP that can be readily modified by PCP-specific PPTases into the holo-BlmI protein, we tested if the holo-BlmI could be aminoacylated in trans, requiring an A domain. Since BlmI has no cognate A domain of its own, we turned our attention to another putative biosynthesis gene cluster we have cloned previously from *Sv* ATCC15003, which encodes at least four NRPS and one PKS modules. We have established that this gene cluster is not clustered with the *blm* locus and is unrelated to BLM biosynthesis. From this gene cluster, we amplified by PCR a 1579 bp fragment encoding an A domain, named Val-A, which we predicted to have a molecular weight of 56,581 and a pI of 7.39. We cloned *val-A* into pET-28a to yield pBS3, in which Val-A would be produced as a fusion protein with a His_6-tag at the N-terminus. Introduction of pBS3 into *E. coli* BL21(DE3) under the standard overexpression conditions recommended by the manufacturer (Novagen) resulted in good overproduction of Val-A, predominantly in soluble form, from which Val-A was purified by affinity chromatography using Ni-NTA resin. The purified Val-A protein was active by the amino acid-dependent ATP-PPi exchange assay (Lee and Lipmann (1970) *Method Emzymol.* 43: 585-602; Ku *et al.* (1997) *Chem. Biol.*, 4: 203-207). Among the 23 amino acids tested, Val-A specifically activated valine, an amino acid that is not required for BLM biosynthesis.

To carry out the aminoacylation in trans, we incubated the purified holo-BlmI and Val-A in vitro in the presence *L*-[^{14}C(U)]valine and ATP (Stachelhaus *et al.* (1996) *Chem. Biol.* 3: 913-921; Weinreb *et al.* (1998) *Biochemistry* 37: 1575-1584). The aminoacylated holo-BlmI-*L*-[^{14}C(U)]valine species was subjected to SDS-PAGE and specific attachment of *L*-[^{14}C(U)]valine to holo-BlmI was determined by autoradiographic analysis. Remarkably, the holo-BlmI was specifically labeled by *L*-[^{14}C(U)]valine in the presence of Val-A, indicative of the formation of the holo-BlmI-S-valine thioester. The in trans aminoacylation between the holo-BlmI and Val-A proteins appeared to be very specific. Neither incubation of *L*-[^{14}C(U)]valine with Val-A, the apo-BlmI, or the holo-BlmI protein alone, nor incubation of *L*-[^{14}C(U)]valine with the Val-A and apo-BlmI proteins, resulted in the detection of ^{14}C-labeled BlmI protein.

Discussion.

Nonribosomal peptides and polyketides are two distinct classes of natural products yet are assembled from amino acids and short carboxylic acids by NRPSs and PKSs, respectively, in strikingly similar strategies (Cane *et al.* (1998) *Science* 282: 63-68).

These fascinating multifunctional enzyme complexes have been classified into two types based on their gene organization and enzyme architecture. Type I enzymes are multifunctional proteins consisting of domains for individual enzyme activities, and type II enzymes are multienzyme complexes consisting of discrete proteins that are largely monofunctional. While both type I and type II PKSs (Fig. 11A and 11C) have been well characterized to account for the vast structural diversities found in polyketide biosynthesis (Hopwood (1997) *Chem Rev.* 97: 2465-2497), all NRPSs studied so far are exclusively the type I modular enzymes (Fig. 11B) (Kleinkauf and von Döhren: H. (1996) *Eur. J. Biochem.* 236: 335-351; Marahiel *et al.* (1997) *Chem. Rev.* 97: 2651-2673; von Döhren *et al.* (1997) *Chem. Rev.* 97: 2675-2705). It is very tempting to speculate the existence of a type II NRPS that, analogous to type II PKS (Shen and Hutchinson (1993) *Science* 262: 1535-1540; Bao *et al.* (1998) *Biochemistry* 37: 8132-8138; Carreras and Khosla (1998) *Biochemistry* 37: 2084-2088), should consist of discrete proteins possessing enzyme activities such as the A (Stachlhaus and Marahiel (1995) *J. Biol. Chem.* 270: 6163-6169), the PCP (Stein and Morris (1996) *J. Biol. Chem.* 271: 15428-15435), or the C (Stachlhaus *et al.* (1998) *J. Biol. Chem.* 273: 22773-22781) domains of type I NRPSs (Fig. 11D). The fact that both the A (Stachlhaus and Marahiel (1995) *J. Biol. Chem.* 270: 6163-6169; Konz *et al.* (1997) *Chem. Biol.* 4: 927-937; Weinreb *et al.* (1998) *Biochemistry* 37: 1575-1584; Mootz and Marahiel (1997) *J. Bacteriol.* 179: 6843-6850) and the PCP (Stachelhaus *et al.* (1996) *Chem. Biol.* 3: 913-921; Weinreb *et al.* (1998) *Biochemistry* 37: 1575-1584l; Pfeifer *et al.* (1995) *Biochemistry* 34: 7450-7459; Haese *et al.* (1994) *J. Mol. Biol.* 243: 116-122; Lambalot *et al.* (1996) *Chem. Biol.* 3: 923-936; Quadri *et al.* (1998) *Biochemistry* 37: 1585-1595; Gehring *et al.* (1996) *Chem. Biol.* 4: 17-24; Ku *et al.* (1997) *Chem. Biol.* 4: 203-207) domains of type I NRPSs can act as independent enzymes supports the hypothesis of a type II NRPS.

We have now cloned and sequenced the *blmI* gene, overproduced and characterized the BlmI protein as a bona fide type II PCP, and demonstrated that holo-BlmI can be aminoacylated by a completely unrelated A domain, providing for the first time genetic and biochemical evidence for a type II NRPS enzyme. We concluded BlmI as a type II PCP based on the following criteria. (1) The deduced amino acid sequence of the *blmI* gene is highly homologous to various PCP domains of known NRPSs, in particular at the signature motif of LGGXS within which the 4'-phosphopantetheine prosthetic group is covalently attached to the serine residue (Marahiel *et al.* (1997) *Chem. Rev.* 97: 2651-2673; Stachelhaus and Marahiel (1995) *FEMS Microbiol. Lett.* 125: 3-14). While the current boundaries for a PCP domain in the literature were defined arbitrarily (Stachelhaus *et al.*

(1996) *Chem. Biol.* 3: 913-921) and varied from one PCP to another, we can now re-define a PCP domain for the type I NRPS as a 90 amino acid peptide with approximately 45 amino acids, each flanking the essential serine residue in the LGGXS (SEQ ID NO:81) motif, in light of this discrete BlmI type II PCP (Fig.9). (2) The *blmI* gene has been successfully expressed in *E. coli*, and fusion of a short peptide to the N-terminus of BlmI dramatically improved its overproduction efficiency. While we cannot exclude the effect of different systems on gene expression, i.e., *E. coli* M15(pREP4)(pBS1) vs. *E. coli* BL21(DE-3)(pBS2), we attribute the increase in expression efficiency to the stability of BlmI as an N-terminal fusion protein instead of the otherwise labile BlmI protein with its native N-terminus. Since BlmI was produced predominantly in the apo-form in *E. coli*, apo-BlmI apparently was not a substrate for the endogenous PPTases, such as EntD or ACP synthase, excluding BlmI as an ArCP or ACP, respectively. EntD and ACP synthase are known to 4'-phosphopantetheinylate apo-ArCP and ACP, respectively, to their holo-forms efficiently (Lambalot *et al.* (1996) *Chem. Biol.* 3: 923-936; Walsh *et al.* (1997) *Curr. Opin. Chem. Biol.* 1: 309-315; Lambalot and Walsh (1995) *J. Biol. Chem.* 270: 24658-24661). (3) The apo-BlmI protein serves as a substrate for PCP-specific PPTases that transfer the 4'-phosphopantetheine moiety from CoA to apo-BlmI to yield the holo-BlmI protein. We have demonstrated this posttranslational modification for BlmI in vivo with the Gsp PPTase (Ku *et al.* (1997) *Chem. Biol.* 4: 203-207) and *in vitro* with the Sfp PPTase (Gehring *et al.* (1998) *Biochemistry* 37: 11637-11650; Lambalot *et al.* (1996) *Chem. Biol.* 3: 923-936; Quadri *et al.* (1998) *Biochemistry* 37: 1585-1595), both of which have been extensively used in preparing holo-PCPs. (4) The specific modification of apo-BlmI by 4'-phosphopantetheinylation has been monitored by HPLC analysis (Fig. 10) (Weinreb *et al.* (1998) *Biochemistry* 37: 1575-1584) and by specific incorporation of [3-^{3}H]-β-alanine *in vivo* (Stachelhaus *et al.* (1996) *Chem. Biol.* 3: 913-921; Ku *et al.* (1997) *Chem. Biol.* 4: 203-207; Epple *et al.* (1998) *J. Bacteriol.* 180: 4950-4954) and of [^{3}H-pantetheine]-CoA *in vitro* (Gehring *et al.* (1998) *Biochemistry* 37: 11637-11650; Lambalot *et al.* (1996) *Chem. Biol.* 3: 923-936; Quadri *et al.* (1998) *Biochemistry* 37: 1585-1595), respectively, into the 4'-phosphopantetheine moiety of the holo-BlmI protein. The identity of BlmI was finally confirmed by MALDI-Tof mass spectral analysis that determined the molecular weight for both the apo- and holo-BlmI proteins.

While individual domains of type I NRPSs can function independently and several A (Stachlhaus and Marahiel (1995) *J. Biol. Chem.* 270: 6163-6169; Konz *et al.*

(1997) *Chem. Biol.* 4: 927-937; Weinreb *et al.* (1998) *Biochemistry* 37: 1575-1584; Mootz and Marahiel (1997) *J. Bacteriol.* 179: 6843-6850) and PCP (Stachelhaus *et al.* (1996) *Chem. Biol.* 3: 913-921; Weinreb *et al.* (1998) *Biochemistry* 37: 1575-1584l; Pfeifer *et al.* (1995) *Biochemistry* 34: 7450-7459; Haese *et al.* (1994) *J. Mol. Biol.* 243: 116-122; Lambalot *et al.* (1996) *Chem. Biol.* 3: 923-936; Quadri *et al.* (1998) *Biochemistry* 37: 1585-1595; Gehring *et al.* (1996) *Chem. Biol.* 4: 17-24; Ku *et al.* (1997) *Chem. Biol.* 4: 203-207) domains have been overproduced, purified, and biochemically characterized, aminoacylation in trans has been successful only between PCPs and their cognate A domains (Stachelhaus *et al.* (1996) *Chem. Biol.* 3: 913-921; Weinreb *et al.* (1998) *Biochemistry* 37: 1575-1584). No aminoacylation between PCP and A domains from different NRPS modules has been observed. These results led to the conclusion that there is a specific protein-protein recognition between the A domain and its cognate PCP (Weinreb *et al.* (1998) *Biochemistry* 37: 1575-1584). Such domain-specific aminoacylation, in fact, should be beneficial in maintaining the fidelity of a type I NRPS by providing additional "gating" against misincorporation of non-specifically activated aminoacyl adenylate into the final peptide product. Since a type II PCP such as BlmI lacks its cognate A domain, we asked if BlmI could be aminoacylated by an unrelated A domain of a type I NRPS. Although we have yet to determine the biochemical role of BlmI in vivo, the fact that the *blmI* gene is located in the middle of the *blm* gene cluster suggests that it may be involved in BLM biosynthesis. To avoid the ambiguity of selecting an A domain that may potentially interact with BlmI in vivo, we preferred not to choose any A domain from the *blm* gene cluster to test if it could aminoacylate BlmI in trans. We reasoned that an A domain that is unrelated to BlmI should come from a gene cluster independent from BLM biosynthesis and should activate an amino acid not required by BLM. We chose Val-A because it satisfied both requirements. Val-A is an A domain of a type I NRPS from a gene cluster we have cloned previously from *Sv* ATCC15003 that has proven to be unrelated to BLM biosynthesis, and it specifically activates valine among the 23 amino acids tested. Remarkably, BlmI was efficiently aminoacylated by Val-A. The valine residue is specifically attached in a thioester linkage to the terminal -SH of the 4'-phosphopantetheine moiety of the holo-BlmI protein, as evidenced by the fact that the apo-BlmI was inactive under the identical conditions.

Aminoacylation of holo-BlmI by Val-A represents the first example in which an A domain aminoacylates a protein other than its cognate PCP domain. Since it has been suggested that an A domain of a type I NRPS can transfer the activated aminoacyl adenylate only to its cognate PCP domain because of the specific protein-protein recognition between

the two domains (Weinreb *et al.* (1998) *Biochemistry* 37: 1575-1584), the fact that BlmI is aminoacylated by Val-A revealed a distinct feature of a type II PCP. It is very tempting to speculate that type II PCPs such as BlmI may have broad intrinsic substrate specificity toward either the aminoacyl adenylate, the A domain, or both. In fact, the latter feature is reminiscent of the type II PKS ACPs, which have been shown to be interchangeable among different PKS complexes (Shen and Hutchinson (1993) *Science* 262: 1535-1540; Bao *et al.* (1998) *Biochemistry* 37: 8132-8138; Carreras and Khosla (1998) *Biochemistry* 37: 2084-2088). The biosynthesis of *D*-alanyl-lipoteichoic acid in *Bacillus suntillis* (Perego *et al.* (1995) *J. Biol. Chem.* 270: 15598-15606) and *Lactobacillus casei* (Debabov *et al.* (1996) 178: 3869-3876) also involves a discrete ACP-like protein, the *D*-alanyl carrier protein, although the latter clearly is structurally and functionally different from PCPs.

The results strongly suggest the existence of a type II NRPS. In fact, we have already identified within the *blm* gene cluster two additional genes, *blmII* and *blmXI* (Fig. 1B), which encode type II C proteins based on sequence analysis (*see* Example 1).

Significance.

All NRPSs known to date are exclusively the type I modular enzymes that are multifunctional proteins consisting of domains, such as A (Stachlhaus and Marahiel (1995) *J. Biol. Chem.* 270: 6163-6169), PCP (Stachelhaus *et al.* (1996) *Chem. Biol.* 3: 913-921), and C (Stachlhaus *et al.* (1998) *J. Biol. Chem.* 273: 22773-22781), for individual enzyme activities (Kleinkauf and von Döhren: H. (1996) *Eur. J. Biochem.* 236: 335-351; Marahiel *et al.* (1997) *Chem. Rev.* 97: 2651-2673; von Döhren *et al.* (1997) *Chem. Rev.* 97: 2675-2705), and control the structural variations of the resulting peptide products by the multiple-carrier thiotemplate mechanism (Cane *et al.* (1998) *Science* 282: 63-68; Stein and Morris (1996) *J. Biol. Chem.* 271: 15428-15435). While individual domains of type I NRPSs can function independently, aminoacylation in trans has been successful only between PCPs and their cognate A domains (Stachelhaus *et al.* (1996) *Chem. Biol.* 3: 913-921; Weinreb *et al.* (1998) *Biochemistry* 37: 1575-1584). We have cloned and sequenced the *blmI* gene, overproduced and characterized the BlmI protein as a bona fide type II PCP, and demonstrated that the holo-BlmI can be aminoacylated by a completely unrelated A domain. Our results provided for the first time the genetic and biochemical evidence to support the hypothesis of a type II NRPS, setting the stage for formulating new research concepts to study peptide biosynthesis. Genetic manipulation of type I NRPS has already been successful in generating novel peptides (Stachlhaus *et al.* (1995) *Science* 269: 69-72). An unprecedented type II NRPS

should shed new light in engineering NRPS proteins, greatly increasing our ability to access peptides with even greater structural diversities.

Materials and methods

General DNA manipulations

Plasmids preparation and DNA extraction were carried out by using commercial kits (Qiagen, Santa Clarita, CA), and all other manipulations were carried out according to standard methods (Sambrook *et al.* (1989) *Molecular cloning: a laboratory manual:* (2nd ed): Cold Spring Harbor Laboratory Press: Cold Spring Harbor: USA). *E. coli* strain DH5α was used as the host for general DNA propagations.

Overexpression *of blmI in E. coli and purification of the BlmI protein*

The *blmI* gene was amplified from *Sv* ATCC15003 by PCR using a forward primer of 5'-CCG CCC ATG GGT GCT CCG CGT GGC GAG CGG ACC CGG CGC-3' (SEQ ID NO:82, the *Nco*I site is underlined) and a reverse primer of 3'-CCT AGA TCT CCG GTC CCG CTC CCC CGT-5' (SEQ ID NO:83, the *Bgl*II site is underlined). In order to create the *Nco*I site, the original starting sequence of "ATG AGC" has been changed to "ATG GGT", which resulted in the change of the second amino acid from serine to glycine. The first five codons of *blmI* were also optimized for overexpression in *E. coli*. The PCR-amplified 0.3 kb *Nco*I-*Bgl*II fragment was cloned into the similar sites of pQE-60 (Qiagen) to form pBS1. Digestion of pBS1 with *Nco*I and *Hind*III and cloning the resulting 0.3 kb *Nco*I-*Hind*III fragment into the same sites of pET-29a (Novagen, Madison, WI) yielded pBS2.

Expressions of *blmI* in *E. coli* M15 (pREP4)(pBS1) and in *E. coli* BL-21(DE-3)(pBS2) and purification of the resulting BlmI protein by affinity chromatography on Ni-NTA resin were carried out under the standard conditions recommended by Qiagen and Novagen, respectively. The incubation temperature was lowered to 30 °C to improve the solubility. The purification of BlmI was monitored by SDS-PAGE on 15% gel. The final pure BlmI protein was desalted on PD-10 column (Sephadex G-25, Pharmacia Biotech, Piscataway, NJ) into 50 mM sodium phosphate buffer, pH 7.8, containing 200 mM NaCl, 10 mM $MgCl_2$, 2 mM dithiothreitol (DTT), 1 mM EDTA, 10% glycerol, and stored at - 80 °C for in vitro assays.

HPLC analysis and MALDI-Tof mass spectral determination

Samples of BlmI (30-70 μg) purified from *E. coli* OG7001(pBS2) or *E. coli* OG7001(pBS2/pDPT-Gsp) were analyzed on a Nova-Pak C18 column (5mm x 10, Waters, Milford, MA) using a Rainin DMAX HPLC unit. The column was developed by a linear gradient of 0-50% acetonitrile in 0.1% trifluoroacetic acid in 25 min, followed by additional 5 min at 50 % acetonitrile, with a flow rate of 0.6 ml/min and detection at 280 nm. MALDI-Tof mass spectral determination was performed on a Bruker Biflex IIII spectrometer at the Facility for Advanced Instrumentation of University of California, Davis.

In vivo labeling of BlmI with [3-^{3}H]-β-alanine

The β-alanine auxotroph *E. coli* strain OG7001 (Epple *et al.* (1998) *J. Bacteriol.* 180: 4950-4954) was transformed with pBS2 and cultured under the same conditions as for *E. coli* BL21(DE3) (Novagen). For co-expression of *blmI* with *gsp*, pDPT-Gsp (Ku *et al.* (1997) *Chem. Biol.* 4: 203-207) was similarly transformed into *E. coli* OG7001(pBS2) and the transformants were cultured in 2xYT (Debabov *et al.* (1996) 178: 3869-3876) in the presence of kanamycin (25 μg/ml) and chloramphenicol (50 μg/ml). For in vivo labeling experiment, cells from 2 ml overnight culture of either *E. coli* OG7001(pBS2) or *E. coli* OG7001(pBS2/pDPT-Gsp) were harvested, washed with M9 minimal medium (Debabov *et al.* (1996) 178: 3869-3876), and re-suspended in 2 ml of M9 minimal medium. The latter were used as seed cultures (20 μl) to inoculate 1 ml M9 medium with kanamycin (25 μg/ml) or kanamycin (25 μg/ml) and chloramphenicol (50 μg/ml) for *E. coli* OG7001(pBS2) or *E. coli* OG7001(pBS2/pDPT-Gsp), respectively. The resulting culture was incubated at 30 °C, 250 rpm to OD_{600nm} 0.6 and to this was added 10 μCi of [3-^{3}H]-β-alanine (50 Ci/mmol, American Radiolabeled Chmicals Inc., St. Louis, MO) with or without IPTG (1 mM). Total proteins were resolved by SDS-PAGE on 15% gels that were Coomassie blue-stained. To determine ^{3}H-labeling of the overproduced holo-BlmI protein, gels were soaked in Amplifier (Amersham, Arlington Heights, Il) for 20 min, dried between two sheets of cellulose membrane (KOH Development Inc., Ann Arbor, MI), and visualized by autoradiography on X-ray films (Fuji Medical Systems, Stamford, CT).

In vitro labeling of BlmI with [^{3}H-pantetheine]-CoA

Expression of *sfp* in *E. coli* MV1190(pUC8-Sfp), purification of the Sfp PPTase to homogeneity, and 4'-phosphopantetheinylation of apo-BlmI by Sfp in vitro were

carried out essentially according to literature procedures (Quadri *et al.* (1998) *Biochemistry* 37: 1585-1595; Nakano *et al.* (1992) *Mol. Gen. Genet.* 232: 313-321). A typical 100 µl assay solution contained 26 µM apo-BlmI, 2.9 µM Sfp, 25 µM [^{3}H-pantetheine]-CoA (0.9 µCi, 40 Ci/mM), 10 mM $MgCl_2$, and 5 mM DTT, in 75 mM MES/NaOAc buffer, pH 6.0. After 30 min incubation at 37 °C, the assays were stopped by addition of 5 µl of bovine serum albumin (0.2 mg/ml) and 0.9 ml of cold 10% (v/v) trichloroacetic acid (TCA). The precipitated proteins were collected by centrifugation at 14,000 rpm, 20 min, 4 °C (Eppendorf 5415C centrifuge), washed with 10% TCA three times, and resolved by SDS-PAGE on 15% gel. The ^{3}H-activity incorporated into holo-BlmI was similarly determined by autoradiography as described for in vivo labeling of holo-Blm with [3-^{3}H]-β-alanine.

Overexpression of val-A in E. coli and purification and assay of the Val-A protein

The *val-A* fragment was amplified from *Sv* ATCC15003 by PCR using a forward primer of 5'-GGA ATT CCA TAT GGG CAC CAC CGT CGC CGC G-3' (SEQ ID NO:84, the *Nde*I site is underlined), and a reverse primer of 3'-GGC AAG CTT GGG ACC GGG CGT GGA GCG C (SEQ ID NO:85, the *Hind*III site is underlined). The PCR-amplified 1.6 kb *Nde*I-*Hind*III fragment was cloned in the similar sites of pET-28a (Qiagen) to yield pBS3. Expression of *val-A* in *E. coli* BL-21(DE-3)(pBS3) and purification of the resulting Val-A protein by affinity chromatography on Ni-NTA resin were carried out under the standard conditions recommended by Novagen.

Amino acid-dependent ATP-PPi assays were performed essentially according to the literature procedures (Ku *et al.* (1997) *Chem. Biol.* 4: 203-207; Lee and Lipmann (1970) *Method Emzymol.* 43: 585-602). A typical 100 µl assay solution contained 180 nM Val-A, 1 mM ATP, 0.1 mM PPi with 0.2 µCi of ^{32}P-PPi (11.75 Ci/mmol, NEN Life Science Products, Inc., Boston, MA), 1 mM $MgCl_2$, 0.1 mM EDTA, and 1 mM *L*-amino acid in 50 mM sodium phosphate buffer, pH 7.8. After 30 min incubation at 30°C, the assays were stopped by addition of 0.9 ml of cold 1% (w/v) activated charcoal in 3% (v/v) perchloric acid. The precipitates were collected on glass fiber filters (2.4 cm, G-4, Fisher, Pittsburgh, PA), washed successively with 10 ml of 0.2 M sodium phosphate buffer, pH 8.0, 4 ml water, and 1 ml of ethanol, and dried in air. The filters were mixed with 7 ml of scintillation fluid (ScintiSafe Gel, Fisher) and counted on a Beckman LS-6800 scintillation counter to determine the radioactivity.

In vitro aminoacylation of holo-BlmI by Val-A

The aminoacylation of holo-BlmI was carried out essentially according to literature methods (Stachelhaus *et al.* (1996) *Chem. Biol.* 3: 913-921; Weinreb *et al.* (1998) *Biochemistry* 37: 1575-1584). A typical 100 µl assay solution contained 180 nM Val-A, 1.5-2.8 µM apo- or holo-BlmI, 35 µM *L*-[^{14}C(U)]-valine (283 mCi/mmol, NEN Life Science Products, Inc., Boston, MA), 5 mM ATP, 10 mM $MgCl_2$, and 5 mM DTT in 75 mM Tris-HCl buffer, pH 8.0. The reactions were started by the addition of ATP and, after incubation at 37 °C for 30 min, were stopped by addition of 0.9 ml of cold 7% (v/v) TCA. The precipitated proteins were collected by centrifugation at 14,000 rpm, 20 min, 4 °C (Eppendorf 5415C centrifuge) and resolved by SDS-PAGE on a 15% gel. The radioactivity incorporated into the holo-BlmI-*L*-[^{14}C(U)]valine species was similarly determined by autoradiography as described for in vivo labeling of holo-BlmI with [3-^{3}H]-β-alanine.

Example 3:

Cloning and characterization of a phosphopantetheinyl transferase from the bleomycin-producing *Streptomyces verticillus* ATCC15003

Multienzymes complexes exist for acyl group activation and transfer reactions in the biogenesis of fatty acids, the polyketide family of natural products (*e.g.* erythromycin, tetracycline), and almost all non-ribosomal peptides (*e.g.* vancomycin, cyclosporin, penicillin). All of these complexes contain one or more small proteins, ~80-100 amino acids long, either as separate subunits or as integrated domains, that function as carrier proteins for the growing acyl chain (acyl-, peptidyl-, and aryl- carrier proteins, abbreviated as ACP, PCP, and ArCP). They are converted from inactive apo-forms to functional holo-forms by the covalent attachment of the 4'-phosphopantetheine moiety of coenzyme A to a conserved serine residue of the carrier-protein substrate. This essential post-translational modification is catalyzed by a family of enzymes known as phosphopantetheinyl transferases (PPTases) (Lambalot et al. *Chem. Biol.* (1996) 3:923-936; Walsh et al. *Curr. Opin. Chem. Biol.* (1997) 1:309-315).

Research in the field of polyketide and non-ribosomal peptide biosynthesis has been hampered by the inability to fully modify and thus convert to the active form some polyketide synthases (PKS) and polypeptide synthetases (NRPS) when overproduced in heterologous hosts, presumably because the host PPTases are unable to effectively modify these overexpressed protein substrates. Our group is currently involved in the

characterization of the gene cluster responsible for the biosynthesis of the antitumor drug bleomycin in *Streptomyces verticillus* ATCC15003. As bleomycin synthetase is a hybrid NRPS/PKS enzyme, we decided to obtain a PPTase from the producing organism in order to use it *in vitro* or *in vivo* by coexpression with the synthetase genes to produce properly modified, active synthetases for our studies.

Results and Discussion

Cloning of the *pttA* gene from *S. verticillus* ATCC15003.

The similarities among PPTases from different organisms are reduced to two short motifs separated by 40-45 residues: (V/I)G(V/I)D, and (F/W)(S/C/T)XKE(A/S)hhK (Lambalot et al. *Chem. Biol.* (1996) 3:923-936; Walsh etal. *Curr. Opin. Chem. Biol.* (1997) 1:309-315). Our previous attempts to amplify PPTase sequences from *S. verticillus* chromosomal DNA using degenerate primers according to the two conserved motifs were unsuccessful (unpublished results), so we decided to narrow our target. PPTases have been classified in two groups, according to their specificity for the carrier-protein substrate: PPTases involved in polyketide/fatty acid biosynthesis use acyl carrier proteins (ACPs) as substrate, while those for non-ribosomal peptide biosynthesis use peptidyl carrier proteins (PCPs) or aryl carrier proteins (ArCPs) (Walsh et al. *Curr. Opin. Chem. Biol.* (1997) 1:309-315). Several "NRPS-type" PPTase sequences were used to screen the databases to look for actinomycete homologues, and four proteins of unknown function were found: NshC from *Streptomyces actuosus* (Li et al. *Gene* (1990) 91:9-17), SC5A7. 23 from *S. coelicolor* (GenBank AL031107), an unnamed protein from *Streptomyces* sp. strain TH1 (Mori et al. *J. Bacteriol.* (1997) 179:5677-5683), and Rv2794c (later renamed PptT (Quadri et al. *Chem. Biol.* (1998) 5:631-645)) from *Mycobacterium tuberculosis* (GenBank AL008967). The alignment of the actinomycete sequences showed the two motifs conserved in all PPTases and an additional motif - the "THC" motif: PXWPXGX$_2$GS(M/L)THCXGY (SEQ ID NO:86), located about 15 amino acids upstream of the (V/I)G(V/I)D motif (SEQ ID NO:87). The "THC" motif is not universally conserved in all PPTases, but it can be detected also in some non-actinomycete PPTases like EntD (Coderre et al. *J. Gen. Microbiol.* (1989) 135:3043-3055). Using a recently developed method of PCR primer design (the CODEHOP strategy (COnsensus-DEgenerate Hybrid Oligonucleotide Primer) (Rose et al. *Nucleic Acids Res.* (1998) 26:1628-1635), two primers were designed around the typical C-terminal PPTase motif (primers KEA-1: 5'-T GCA GCA GAA CAG GAG GCK NYC CCA

NKG-3' (SEQ ID NO:88) and KEA-2: 5'-TG GGT CAG CGG GTA CCA NRC YTT RWA-3' (SEQ ID NO: 89, H=C+A, N=A+C+T+G, Y=C+T, K=G+T, R=A+G, W=T+A)), and one primer was designed from the "THC" motif (primer THC: 5'-C GGC ATG GTC GGC TCC HTN ACN CAY TG-3', SEQ ID NO:90, H=C+A, N=A+C+T+G, Y=C+T, K=G+T, R=A+G, W=T+A); this motif is not universally conserved in PPTases of all organisms). Using *S. verticillus* chromosomal DNA as template, no amplification product was detected using the THC and the KEA-1 primers. The set of primers THC/KEA-2 successfully amplified a single band of the expected size (about 250 bp), which was gel-purified and cloned. Eight individual clones were sequenced, and all of them resulted to be identical (except differences due to primer utilization) and highly similar to the putative actinomycete PPTases. The PCR fragment was used as a probe to screen a *S. verticillus* genomic library by colony hybridization. Of the 10,000 colonies screened, 25 positive clones were identified, and then confirmed by Southern analysis to contain the same 4. 6-kb *Bam*HI hybridizing band. The 4. 6-kb DNA fragment was subcloned, and the nucleotide sequence of a 1,761-bp *Bam*HI-*Sal*I region was determined (SEQ ID NO. 3).

Sequence analysis of the *pptA* locus.

The sequence of the 1,761-bp *Bam*HI-*Sal*I fragment was analyzed for coding regions by using the CODONPREFERENCE and TESTCODE programs of the GCG package (Genetics Computer Group, Madison, Wisconsin). Two complete ORFs (*pptA, orf3*) and two incomplete ORFs (*orf1, orf4*) were identified within the sequenced region (Figure 13). The first ORF from left to right (designated *orf1*) starts out of the analyzed area and ends with a TGA codon at position 248 of the sequenced fragment. Comparison of the deduced product of *orf1* with proteins in databases showed similarities with Rv2795c from *Mycobacterium tuberculosis* (GenBank AL008967) and SC5A7. 22 from *S. coelicolor* (GenBank AL031107), both of unknown function. The second ORF, *pptA*, contains the sequence amplified by PCR and used for the cloning of this locus. It comprises 741 nucleotides, starting with a GTG codon (position 245) which is coupled to the stop codon of *orf1*, and ending with a TAA codon. The starting codon of *pptA* is preceded by a potential ribosomal binding site (RBS), GGGAG. The overall (76. 6%) and third codon position (93. 9%) G+C contents and the codon usage of *pptA* are similar to those found in other *Streptomyces* genes, with the exception of the stop codon (TAA), which is most uncommon in this group of organisms (Wright et al. *Gene* (1992) 113:55-65). The *pptA* gene encodes a protein of 246 amino acids with a predicted molecular mass of 25,619 Da and a pI of 4. 76,

which contains the conserved PPTase motifs. Databases searches with PptA showed significant similarities to the putative actinomycete PPTases (39-52%/48-61% identity/similarity) and to confirmed bacterial PPTases such as EntD from *E. coli* (17%/24% identity/similarity) (Lambalot et al. *Chem. Biol.* (1996) 3:923-936). The third ORF, *orf3*, is separated from *pptA* by an apparently noncoding DNA region of 153 bp, and it is transcribed in opposite and convergent direction with respect to *orf1-pptA*. The gene *orf3* comprises 240 nucleotides, starting with an ATG codon (position 1358) and ending with TGA. The starting codon of *orf3* is preceded by the sequence GAAGG, a potential RBS. The deduced product of *orf3* encodes a protein of 79 amino acids with a predicted mass of 7,555 Da and a pI of 7. 17. The Orf3 protein shows similarities to the N-terminal region of SC5H1. 35c, a protein of unknown function from *S. coelicolor* (GenBank AL049863). Analysis of Orf3 with the SignalP program (Nielsen et al. *Protein Engineer.* (1997) 10:1-6) predicts an N-terminal signal peptide which would be cleaved between residues 27 and 28 (ALA-DS), suggesting that the mature protein (52 amino acids, 5,099 Da, pI 4. 31) would be secreted. Between *orf3* and *orf4* there is an apparently noncoding region of 251 nucleotides. The *orf4* gene is transcribed in opposite and divergent direction with respect to *orf3*. It starts with an ATG codon at position 1610, preceded by a potential RBS (GGAGG), and ends out of the sequenced fragment. The deduced protein product (50 amino acids) of the incomplete *orf4* contains a potential NAD/FAD binding motif, $GXGX_2GX_3GX_6G$ (Scrutton et al. *Nature* (1990) 343:38-43), showing low similarities to diverse oxidoreductases.

Heterologous expression and biochemical characterization of PptA.

In order to test if *pptA* actually encodes a functional PPTase, we decided to overproduce and purify the PptA protein, and assay its catalytic competence on putative substrate proteins or domains. The *pptA* coding sequence was amplified by PCR and cloned into the T5-promoter-based pQE-70 vector, yielding plasmid pQEPPT, in such a way that a hexahistidine tag would be added at the C-terminus of the protein. Expression of the pQEPPT construct in *E. coli* M15(pREP4) resulted in the overproduction of soluble His-tagged PptA which was readily purified by affinity chromatography on Ni-NTA agarose under non-denaturing conditions (FIGURE). Because *pptA* belongs, by sequence similarity, to the subfamily of PPTases involved in nonribosomal peptide synthesis, we first assayed its activity using two different apo-PCPs as protein substrates. The first one, BlmI, has been previously characterized in our laboratory as a discrete peptidyl carrier protein, or type II PCP, whose gene is found within the bleomycin-biosynthesis gene cluster of *S. verticillus*

(Du et al. *Chem. Biol.* (1999) 6:507-517). For the second PCP substrate we used BlmX, a bimodular NRPS protein encoded in the same cluster (Fig. 2), as a source of a type I PCP, i. e. a PCP included in a multidomain NRPS. For the production of this type I PCP, we amplified by PCR a 1,898 bp fragment encoding the adenylation and PCP domains from the second module of BlmX. This DNA fragment was cloned into pMAL-c2x to yield pMAL1617, in which the type I PCP would be produced as a maltose-binding protein (MBP) fusion, MBlmX-2, with a predicted molecular mass of 108. 5 kDa. Introduction of pMAL1617 in *E. coli* TB1 resulted in good overproduction of MBlmX-2, about 40% soluble, which was purified by affinity chromatography using amylose resin. To test the PPTase activity, we incubated the purified PptA with BlmI and MBlmX-2 as putative protein substrates in the presence of (^{3}H)-(pantetheinyl)-CoASH, and the tritiated products were subjected to SDS electrophoresis and autoradiography. The well-characterized PPTase Sfp from *B. subtilis*; which exhibits a broad specificity for its protein substrate (Quadri et al. *Biochemistry* (1998) 37:1585-1595), was included as a positive control. In these experiments PptA exhibited a robust phosphopantetheinylation activity on both BlmI and MBlmX-2. Having demonstrated that PptA does in fact have PPTase activity on both type I and type II PCP substrates from nonribosomal peptide synthetases, we then proceeded to test two different acyl-carrier proteins (ACPs) as potential substrates. The first one, BlmVIII, is a monomodular multidomain polyketide synthase (PKS) which is encoded in the bleomycin-biosynthesis gene cluster of *S. verticillus* (Fig. 2). BlmVIII contains an ACP domain at its C-terminus, that is a type I ACP. For the second ACP substrate we used TcmM, a type II acyl carrier protein involved in the biosynthesis of the aromatic polyketide tetracenomycin C in *S. glaucescens* (Shen et al. *J. Bacteriol.* (1992) 174:3818-3821; Bao et al. *Biochemistry* (1998) 37: 8132-8138). For the production of TcmM, its coding sequence was transferred from a construct previously made in pET-22b (Gehring et al. *Chem. Biol.* (1997) 4:17-24) into the pET-28a vector to yield pET28a-TcmM, in such a way that a hexahistidine tag should be added at both the N-terminus and the C-terminus of the protein. Plasmid pET28a-TcmM was introduced into *E. coli* BL21(DE3), and TcmM was easily purified by affinity chromatography using Ni-NTA resin. In vitro phosphopantetheinylation assays were performed as before, but using BlmVIII and TcmM as protein substrates, and PptA was able to posttranslationally modified both ACP substrates.

The *pptA* gene is not clustered to the bleomycin-biosynthesis locus.

Some bacterial PPTase genes have been found clustered, or close, to their respective "partner" NRPS genes: *entD* {enterobactin (Coderre et al. *J. Gen. Microbiol.* (1989) 135:3043-3055)}, *sfp* {surfactin (Cosmina et al. *Mol. Microbiol.* (1993) 8:821-831)}, *gsp* {gramicidin (Borchert et al. *J. Bacteriol.* (1994) 176:2458-2462)}, *bli* {bacitracin (Gaidenko et al. *Biotechnologia* (1992) 13-19)}, *lpa-14* {iturin (Huang et al. *J. Ferment. Bioeng.* (1993) 76:445-450)}. To test the possible clustering of *pptA* to the bleomycin-biosynthesis (*blm*) locus, PCR reactions were performed using the THC/KEA-2 primers on several overlapping cosmid clones spanning the *blm* locus plus 30-40 kb upstream and downstream of its putative limits. No amplification product could be obtained in these reactions, showing that the *pptA* gene is not clustered with the *blm* locus.

Discussion

It has been suggested that in organisms containing multiple phosphopantetheine-requiring pathways, each pathway has its own posttranslational modifying activity (Walsh et al. *Curr. Opin. Chem. Biol.* (1997) 1:309-315). Our group has found that *S. verticillus* ATCC15003 contains several PKS and NRPS gene clusters, one of them being responsible for bleomycin production (a hybrid NRPS/PKS system) (Shen et al. *Bioorg. Chem.* (1999) 27:155-171; Du et al. *Chem. Biol.* (1999) 6:507-517). This suggested that the gene encoding the PPTase for the BLM NRPS could be also clustered, or close, to the NRPS genes. However, we have not found this gene after sequencing almost the whole *blm* NRPS locus. Because having this gene could be important for us in order to express functional NRPS modules from the *blm* cluster, we decided to clone the PPTase gene. Additionally, if the "one NRPS cluster - one PPTase" hypothesis was true, it seemed possible to use PPTase sequences as a new kind of probe to clone novel NRPS clusters.

We know that in *S. verticillus* there are several NRPS locus (maybe four), so we expected several "PCP-type" PPTases. However we have amplified only one, and it does not seem to be closely linked to any of the NRPS loci. Interestingly in the actinomycete *Mycobacterium tuberculosis*, whose genome is fully sequenced, there is only one PCP-type PPTase gene, which is not clustered with any of the two NRPS loci present in this organism (Quadri et al, *Chem. Biol.* (1998) 5:631-645). These and other indirect evidences suggest that the idea of cluster-specific PPTases is not the general rule at all but most probably the exception, especially in organisms containing multiple NRPS clusters. And there are strong evidences that at least some PCP-type PPTases can posttranslationally modify PCPs from

different clusters and even different organisms (Quadri et al, *Chem. Biol.* (1998) 5:631-645; Gehring et al, *Biochemistry* (1998) 37:11637-11650). It is most likely that there is only one PCP-type PPTase in *S. verticillus* and that its gene is not necessarily clustered to any of the NRPS loci.

Biochemical characterization of the purified PptA protein confirmed not only its PPTase activity but also its broad specificity, comparable to that of Sfp. Different apo-PCPs (type I and type II) and a type-I apo-ACP from the bleomycin synthetase, and the type-II apo-ACP from the tetracenomycin PKS of *Streptomyces glaucescens* were efficiently used as substrates by PptA. These results suggest PptA as a good candidate for heterologous coexpression with NRPS and PKS genes to overproduce active holo-synthase enzymes.

It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

CLAIMS

What is claimed is:

1. An isolated nucleic acid comprising a nucleic acid selected from the group consisting of

a nucleic acid encoding any one of *Blm* open reading frames (ORFs) 8 through 41;

a nucleic acid encoding a polypeptide encoded by any one of *Blm* open reading frames (ORFs) 8 through 41; and

a nucleic acid amplified by polymerase chain reaction (PCR) using any one of the primer pairs identified in Table II and the nucleic acid of a bleomycin-producing organism as a template.

2. The isolated nucleic acid of claim 1, wherein said nucleic acid comprises a nucleic acid encoding at least two open reading frames selected from the group consisting of *Blm* open reading frames 8 through 41.

3. The isolated nucleic acid of claim 1, wherein said nucleic acid comprises a nucleic acid encoding at least three open reading frames selected from the group consisting of *Blm* open reading frames 8 through 41.

4. The isolated nucleic acid of claim 1, wherein said nucleic acid comprises a nucleic acid encoding a C domain lacking one or more His residues of the conserved HHxxxDG active site for transpeptidation.

5. The isolated nucleic acid of claim 1, wherein said nucleic acid comprises a nucleic acid encoding a protein encoded by a gene selected from the group consisting of *blmI, blmII,* and *blmXI.*

6. An isolated nucleic acid comprising a nucleic acid encoding a module comprising two or more catalytic domains of a protein encoded by a nucleic acid of a bleomycin gene cluster wherein said catalytic domains are selected from the group consisting of a condensation (C) domain, an adenylation (A) domain, a peptidyl carrier protein (PCP) domain, a condensation/cyclization domain (Cy), an acyl-carrier protein (ACP)-like domain,

an oxidization domain (Ox), a ketoacyl synthase (KS) domain , an acetyl transferase (AT) domain, a ketoreductase (KR) domain, and a methyltransferase (MT) domain.

7. The isolated nucleic acid of claim 6, wherein said nucleic acid comprises a nucleic acid encoding one or more proteins comprising a module selected from the group consisting of NRPS-0, NRPS-1, NRPS-2, NRPS-3, NRPS-4, NRPS-5, NRPS-6, NRPS-7, NRPS-7, NRPS-9, and PKS.

8. The isolated nucleic acid of claim 7, wherein said nucleic acid comprises an open reading frame from SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

9. An isolated nucleic acid comprising a nucleic acid encoding a protein encoded by a gene from a BLM gene cluster.

10. The nucleic acid of claim 9, wherein said nucleic acid comprises a nucleic acid encoding a protein encoded by a gene selected from the group consisting of *blmI, blmII, and blmXI.*

11. The nucleic acid of claim 9, wherein said nucleic acid comprises a nucleic acid encoding a protein encoded by a gene selected from the group consisting of *blmIII, blmIV, blmV, blmVI, blmVII, blmIX, and blmX.*

12. The nucleic acid of claim 9, wherein said nucleic acid comprises a nucleic acid encoding a protein encoded by *blmVIII.*

13. The nucleic acid of claim 9, wherein said nucleic acid comprises a nucleic acid selected from the group consisting of *blmI, blmII, and blmXI.*

14. The nucleic acid of claim 9, wherein said nucleic acid comprises a nucleic acid selected from the group consisting of *blmIII, blmIV, blmV, blmVI, blmVII, blmIX, and blmX.*

15. The nucleic acid of claim 9, wherein said nucleic acid comprises *blmVIII.*

16. An isolated nucleic acid comprising a nucleic acid that encodes a protein comprising at least one catalytic domain selected from the group consisting of a condensation (C) domain, an adenylation (A) domain, a peptidyl carrier protein (PCP)

domain, a condensation/cyclization domain (Cy), an acyl-carrier protein (ACP)-like domain, an oxidization domain (Ox), a ketoacyl synthase (KS) domain , an acetyl transferase (AT) domain, a ketoreductase (KR) domain, and a methyltransferase (MT) domain, and that hybridizes to a nucleic acid selected from the group consisting of *orf8*, *orf9*, *orf10*, *orf11*, *orf12*, *orf13*, *orf14*, *orf15*, *orf15*, *orf16*, *orf17*, *orf18*, *orf19*, *orf20*, *orf21*, *orf22*, *orf23*, *orf24*, *orf25*, *orf26*, *orf27*, *orf28*, *orf29*, *orf30*, *orf31*, *orf32*, *orf33*, *orf34*, *orf35*, *orf36*, *orf37*, *orf38*, *orf39*, *orf40*, and *orf41* under stringent conditions.

17. The nucleic acid of claim 16, wherein said isolated nucleic acid comprises a nucleic acid encoding a module.

18. The nucleic acid of claim 16, wherein said isolated nucleic acid comprises a nucleic acid encoding a BLM gene.

19. An isolated nucleic acid comprising a nucleic acid selected from the group consisting of consisting of *orf8*, *orf9*, *orf10*, *orf11*, *orf12*, *orf13*, *orf14*, *orf15*, *orf15*, *orf16*, *orf17*, *orf18*, *orf19*, *orf20*, *orf21*, *orf22*, *orf23*, *orf24*, *orf25*, *orf26*, *orf27*, *orf28*, *orf29*, *orf30*, *orf31*, *orf32*, *orf33*, *orf34*, *orf35*, *orf36*, *orf37*, *orf38*, *orf39*, *orf40*, and *orf41*, or an allelic variant thereof.

20. The nucleic acid of claim 19, wherein said nucleic acid comprises a nucleic acid that is a single nucleotide polymorphism (SNP) of a nucleic acid selected from the group consisting of consisting of *orf8*, *orf9*, *orf10*, *orf11*, *orf12*, *orf13*, *orf14*, *orf15*, *orf15*, *orf16*, *orf17*, *orf18*, *orf19*, *orf20*, *orf21*, *orf22*, *orf23*, *orf24*, *orf25*, *orf26*, *orf27*, *orf28*, *orf29*, *orf30*, *orf31*, *orf32*, *orf33*, *orf34*, *orf35*, *orf36*, *orf37*, *orf38*, *orf39*, *orf40*, and *orf41*.

21. An isolated gene cluster comprising open reading frames encoding polypeptides sufficient to direct the assembly of a bleomycin.

22. An isolated multi-functional protein complex comprising both a polyketide synthase (PKS) and a peptide synthetase (NRPS).

23. An isolated nucleic acid encoding a multi-functional protein complex comprising both a polyketide synthase (PKS) and a peptide synthetase (NRPS).

24. An isolated polypeptide comprising a catalytic domain encoded by a nucleic acid of a bleomycin gene cluster wherein said nucleic acid comprises a nucleic acid selected from the group consisting of

a nucleic acid encoding any one of *Blm* open reading frames (ORFs) 8 through 41; and

a nucleic acid amplified by polymerase chain reaction (PCR) using any one of the primer pairs identified in Table II.

25. The polypeptide ofclaim 25, wherein said polypeptide comprises an enzymatic domain selected from the group consisting of a condensation (C) domain, an adenylation (A) domain, a peptidyl carrier protein (PCP) domain, a condensation/cyclization domain (Cy), an acyl-carrier protein (ACP)-like domain, an oxidization domain (Ox), a ketoacyl synthase (KS) domain , an acetyl transferase (AT) domain, a ketoreductase (KR) domain, and a methyltransferase (MT) domain.

26. The polypeptide claim 25, wherein the nucleic acid of a bleomycin gene cluster comprises a nucleic acid encoding at least two open reading frames selected from the group consisting of *Blm* open reading frames 8 through 41.

27. The polypeptide claim 25, wherein said nucleic acid of a bleomycin gene cluster comprises a nucleic acid encoding at least three open reading frames selected from the group consisting of *Blm* open reading frames 8 through 41.

28. The polypeptide claim 25, wherein said polypeptide comprises a C domain lacking one or more His residues of the conserved HHxxxDG active site for transpeptidation.

29. The polypeptide claim 25, wherein said polypeptide is a polypeptide encoded by a gene selected from the group consisting of *blmI, blmII,* and *blmXI.*

30. An isolated polypeptide comprising a module comprising two or more catalytic domains of a protein encoded by a nucleic acid of a bleomycin gene cluster wherein said catalytic domains are selected from the group consisting of a condensation (C) domain, an adenylation (A) domain, a peptidyl carrier protein (PCP) domain, a condensation/cyclization domain (Cy), an acyl-carrier protein (ACP)-like domain, an

oxidization domain (Ox), a ketoacyl synthase (KS) domain , an acetyl transferase (AT) domain, a ketoreductase (KR) domain, and a methyltransferase (MT) domain.

31. The polypeptide of claim 30, wherein said polypeptide comprises a module selected from the group consisting of NRPS-0, NRPS-1, NRPS-2, NRPS-3, NRPS-4, NRPS-5, NRPS-6, NRPS-7, NRPS-7, NRPS-9, and PKS.

32. An isolated polypeptide encoded by a gene from a BLM gene cluster.

33. The polypeptide of claim 32, wherein polypeptide is encoded by a gene selected from the group consisting of *blmI, blmII, and blmXI.*

34. The polypeptide of claim 32, wherein said nucleic acid comprises a nucleic acid encoding a protein encoded by a gene selected from the group consisting of *blmIII, blmIV, blmV, blmVI, blmVII, blmIX, and blmX.*

35. The polypeptide of claim 32, wherein polypeptide is encoded by *blmVIII.*

36. An isolated polypeptide comprising a module wherein said module is specifically bound by an antibody that specifically binds to a BLM module selected from the group consisting of NRPS-0, NRPS-1, NRPS-2, NRPS-3, NRPS-4, NRPS-5, NRPS-6, NRPS-7, NRPS-7, NRPS-9, and PKS.

37. The polypeptide of claim 36, wherein said polypeptide is specifically bound by an antibody that specifically binds to a polypepide encoded by a gene selected from the group consisting of of *blmI, blmII, blmXI, blmIII, blmIV, blmV, blmVI, blmVII, blmIX, blmX,* and *blmVIII.*

38. An isolated polypeptide comprising a polypeptide encoded an open reading frame of a nucleic acid selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3, or an allelic variant thereof.

39. The polypeptide of claim 38, wherein said nucleic acid comprises a single nucleotide polymorphism (SNP) of an open reading of a nucleic acid selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3.

40. An expression vector comprising a nucleic acid of any one of claims 1 through 23.

41. A host cell transformed with an expression vector of claim 40.

42. The host cell of claim 41, wherein said cell is transformed with an exogenous nucleic acid comprising a gene cluster encoding polypeptides sufficient to direct the assembly of a bleomycin or bleomycin analog.

43. The cell of claim 41, wherein said cell is a bacterial cell.

44. The cell of claim 43, wherein said cell is a *Streptomyces* cell.

45. The cell of claim 41, wherein said cell is a eukaryotic cell.

46. A method of chemically modifying a biological molecule, said method comprising contacting a biological molecule that is a substrate for a polypeptide encoded by one or more bleomycin biosynthesis gene cluster open reading frames with the polypeptide encoded by one or more bleomycin biosynthesis gene cluster open reading frames, whereby said polypeptide chemically modifies said biological molecule.

47. The method of claim 46, wherein said method comprising contacting said biological molecule with at least two different polypeptides encoded by *blm* gene cluster open reading frames.

48. The method of claim 46, wherein said method comprising contacting said biological molecule with at least three different polypeptides encoded by *blm* gene cluster open reading frames.

49. The method of claim 46, wherein said contacting is in a host cell.

50. The method of claim 49, wherein said host cell is a bacterium.

51. The method of claim 46, wherein said contacting *ex vivo*.

52. The method of claim 46, wherein said biological molecule is an endogenous metabolite produced by said host cell.

53. The method of claim 46, wherein said biological molecule is an exogenous supplied metabolite.

54. The method of claim 46, wherein said host cell is a eukaryotic cell.

55. The method of claim 54, wherein said eukaryotic cell is selected from the group consisting of a mammalian cell, a yeast cell, a plant cell, a fungal cell, and an insect cell.

56. The method of claim 46, wherein said biological molecule is an amino acid and said polypeptide is a peptide synthetase.

57. The method of claim 46, wherein said polypeptide is a methyl transferase.

58. A method of coupling a first amino acid to a second amino acid, said method comprising contacting the first and second amino acid with a recombinantly expressed bleomycin nonribosomal peptide synthetase (NRPS).

59. The method of claim 64, wherein said NRPS is selected from the group consisting of NRPS-5, NRPS-4, NRPS-3, NRPS-9, NRPS-8, and NRPS-7.

60. The method of claim 64, wherein said NRPS is selected from the group consisting of NRPS-6, NRPS-2, NRPS-1, and NRPS-0.

61. The method of claim 64, wherein said contacting is in a host cell.

62. A method of coupling a first fatty acid to a second fatty acid, said method comprising contacting the first and second fatty acids with a recombinantly expressed bleomycin polyketide synthase (PKS).

63. The method of claim 62, said contacting is in a host cell.

64. A method of producing a bleomycin or bleomycin analog, said method comprising:

providing a cell transformed with an exogenous nucleic acid comprising a bleomycin gene cluster encoding polypeptides sufficient to direct the assembly of said bleomycin or bleomycin analog;

culturing the cell under conditions permitting the biosynthesis of bleomycin or bleomycin analog; and

isolating said bleomycin or bleomycin analog from said cell.

65. An isolated nucleic acid comprising a nucleic acid encoding a phosphopantetheinyl transferase said nucleic acid encoding a phosphopantetheinyl transferase being selected from the group consisting of:

a nucleic acid encoding the protein encoded by the nucleic acid of SEQ ID NO:3;

a nucleic acid amplified by polymerase chain reaction (PCR) using primers that specifically amplify ORF 41 (primers: SEQ ID NO:71 and SEQ ID NO:72) and *Streptomyces* nucleic acid as a template;

a nucleic acid encoding a polypeptide having phosphopantetheinyl transferase activity where said nucleic acid specifically hybridizes to the nucleic acid of SEQ ID NO: 3 under stringent conditions.

66. The nucleic acid of claim 65, said nucleic acid comprising a nucleic acid of SEQ ID NO:3.

67. A polypeptide comprising a phosphopantetheinyl transferase encoded by SEQ ID NO:3.

68. A vector comprising the nucleic acid of claim 66.

69. A cell transfected with the vector of claim 68.

70. A method of converting an apo-carrier protein to a holo-carrier protein comprising reacting said apo-carrier protein with a recombinant phosphopantetheinyl transferase encoded by SEQ ID NO:3 and coenzyme A thereby producing a holo-carrier protein.

71. A cell comprising a modified bleomycin gene cluster nucleic acid, said cell producing elevated amounts of bleomycin as compared to the wild type cell.

72. The cell of claim 71, wherein said cell overexpresses a resistance gene from the bleomycin bene cluster.

73. The cell of claim 72, wherein said resistance gene is a gene listed in Table III.

FIG. 1A

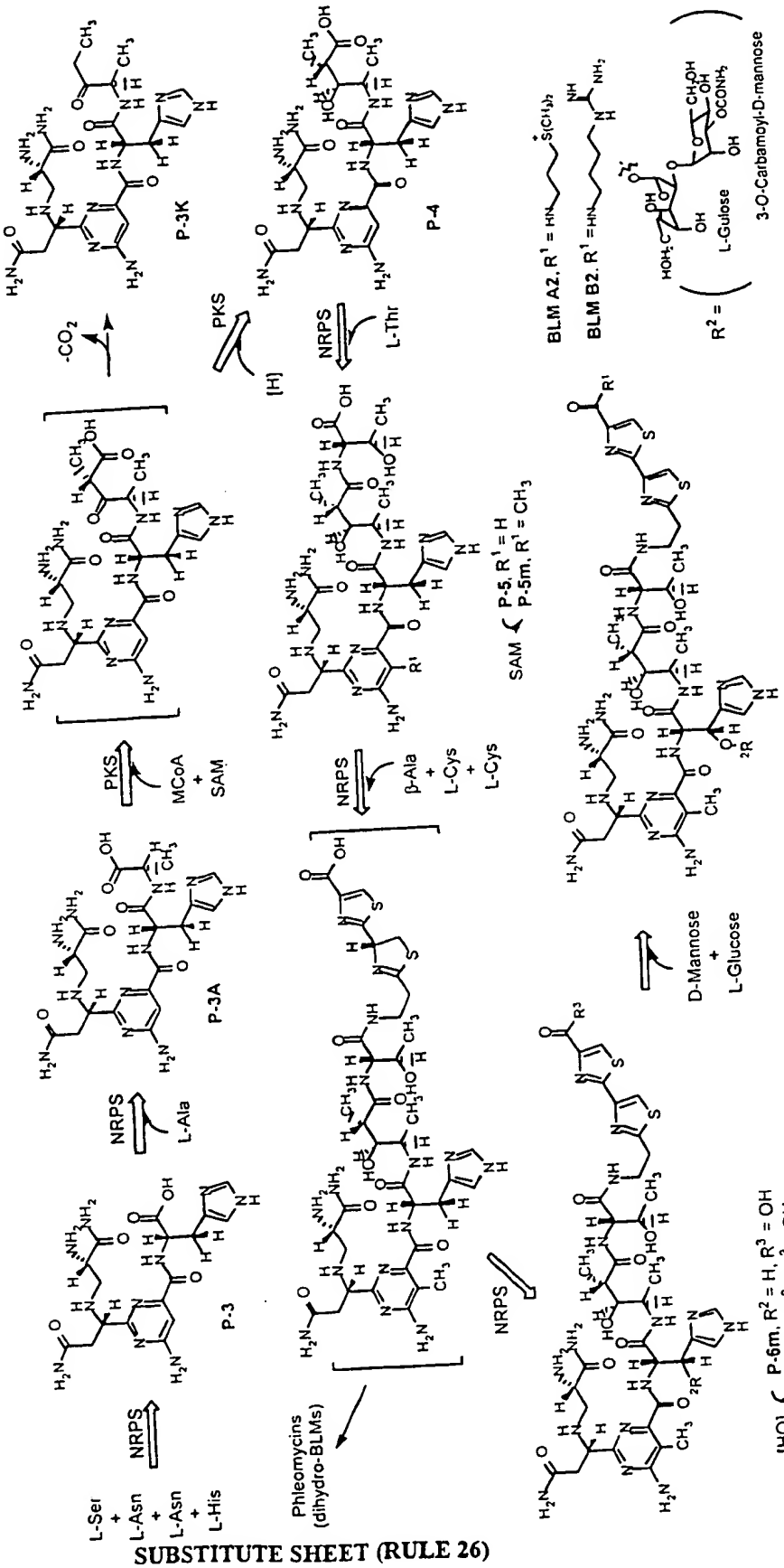

L-Ser
+
L-Asn
+
L-Asn
+
L-His
NRPS
P-3
NRPS
L-Ala
P-3A
PKS
MCoA
+
SAM
-CO2
P-3K
PKS
[H]
NRPS
L-Thr
P-4
Phleomycins
(dihydro-BLMs)
NRPS
β-Ala
+
L-Cys
+
L-Cys
SAM
P-5, R1 = H
P-5m, R1 = CH3
NRPS
D-Mannose
+
L-Glucose
[HO]
P-6m, R2 = H, R3 = OH
P-6mo, R2 = R3 = OH
Terminal amines
BLM aglycone, R2 = OH, R3 = terminal amines
BLM A2, R1 =
BLM B2, R1 =
R2 =
L-Gulose
3-O-Carbamoyl-D-mannose

FIG. 1B

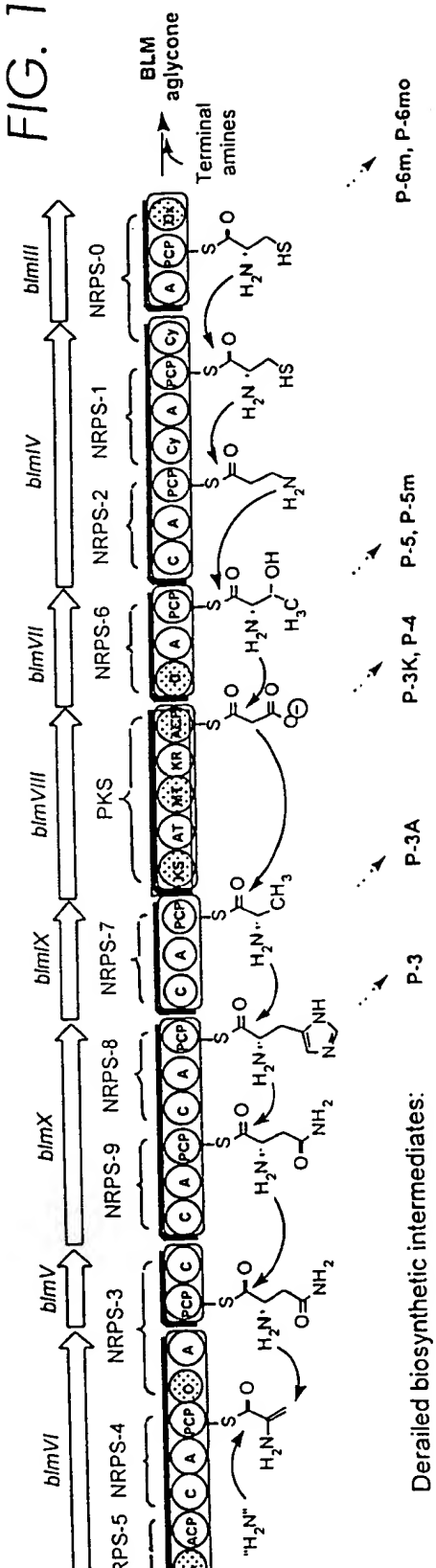

blmVI
blmV
blmX
blmIX
blmVIII
blmVII
blmIV
blmIII
NRPS-5
NRPS-4
NRPS-3
NRPS-9
NRPS-8
NRPS-7
PKS
NRPS-6
NRPS-2
NRPS-1
NRPS-0
BLM aglycone
Terminal amines
"H2N"
Derailed biosynthetic intermediates:
P-3
P-3A
P-3K, P-4
P-5, P-5m
P-6m, P-6mo

FIG. 2

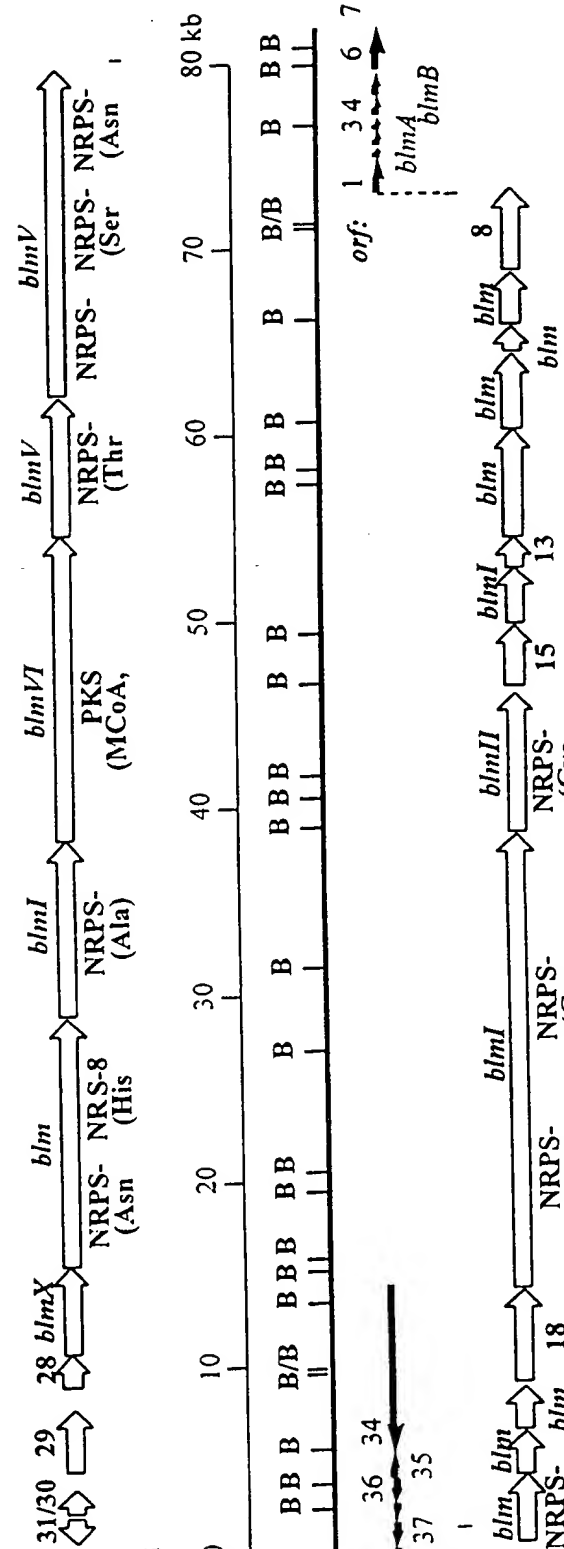

blmV
NRPS- (Asn
blmX
blm
NRPS-8 (His
blmI
NRPS- (Ala)
blmVI
PKS (MCoA,
blmV
NRPS- (Thr
blmV
NRPS- (Ser
NRPS- (Asn
80 kb
orf: 1 3 4 6 7
blmA
blmB
orf:40
blm
NRPS- (β-Ala)
blmI
NRPS- (Cys
blmII
NRPS- (Cys
blmJ
blm
blm
blm
blm

FIG. 3A

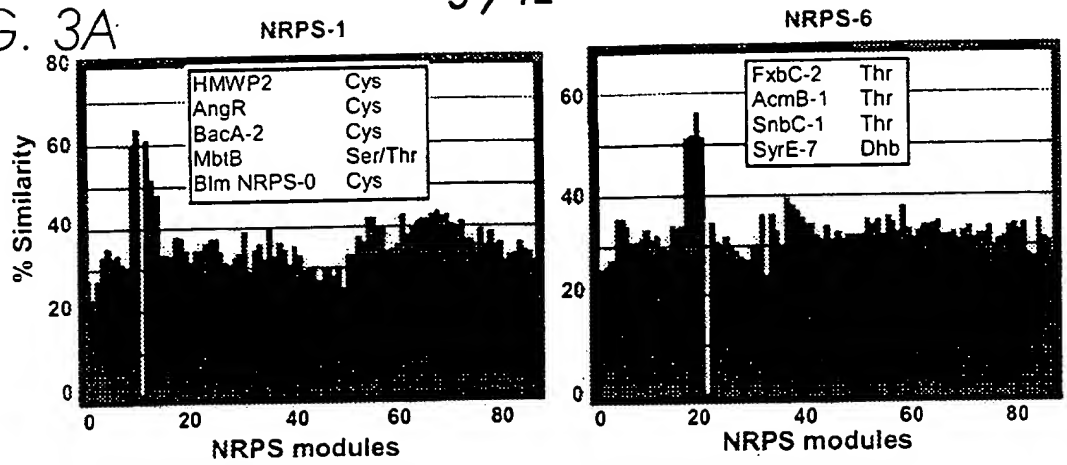


FIG. 3B

NRPS module	Substrate	Residues (PheA numbering) (16) 236	239	278	299	301	322	330	331
HMWP2	Cys	L	Y	N	M	S	M	I	W
AngR	Cys	L	Y	N	M	S	M	I	W
BacA-2	Cys	L	Y	N	L	S	L	I	W
MbtB	Ser/Thr	M	L	N	A	G	L	V	H
Bim NRPS-0	**Cys**	L	Y	H	L	G	L	P	W
Bim NRPS-1	**Cys**	L	Y	N	L	S	L	I	W
SyrE-7	Dhb	F	W	N	V	G	M	V	H
AcmB-1	Thr	F	W	N	V	G	M	V	H
SnbC-1	Thr	F	W	N	I	G	M	V	H
FxbC-2	Thr	F	W	N	V	G	M	V	H
Bim NRPS-6	**Thr**	F	W	S	V	G	M	I	H

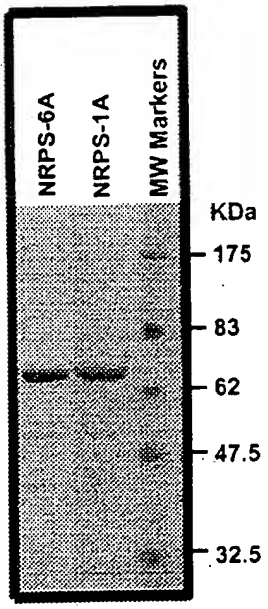


FIG. 3C

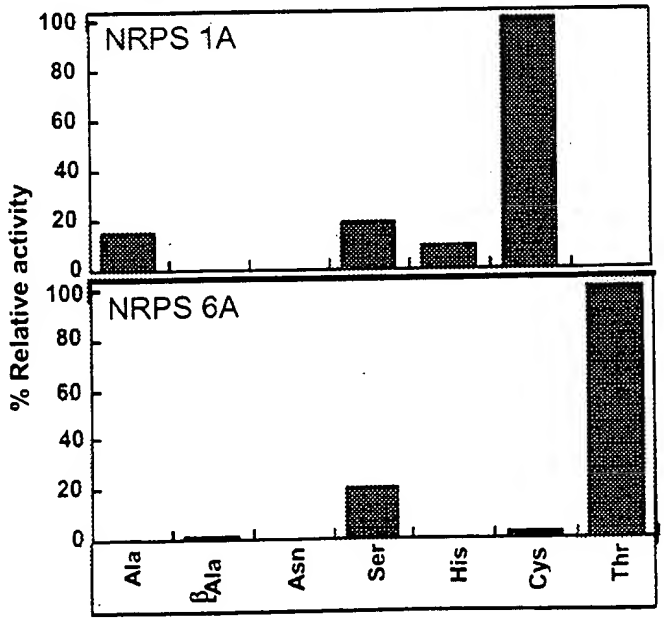
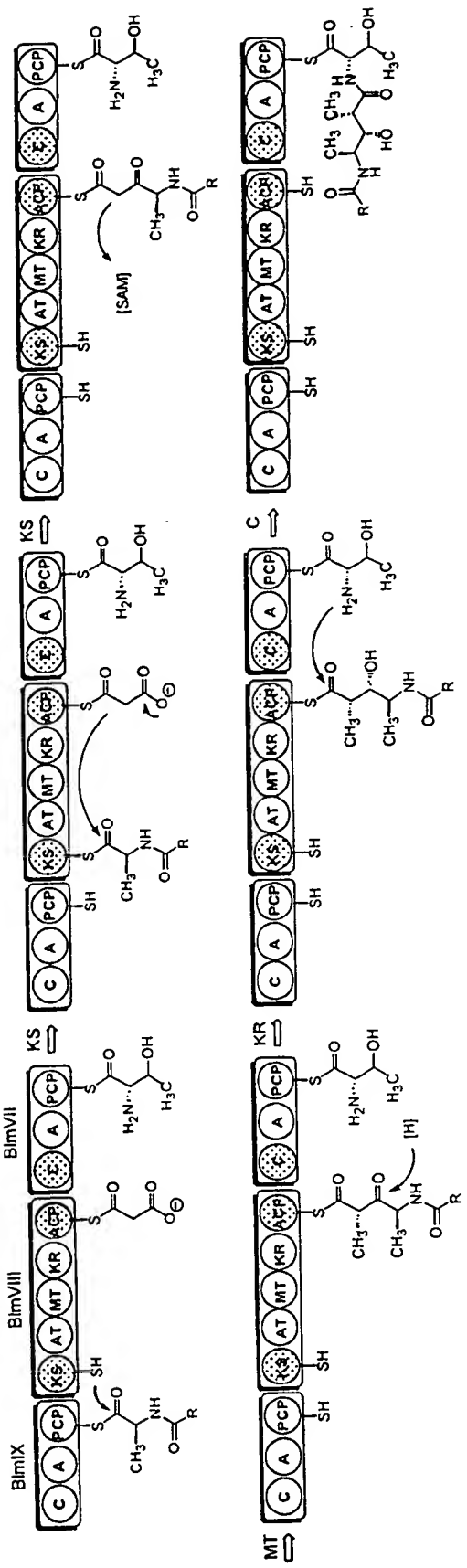


FIG. 3D

FIG. 4



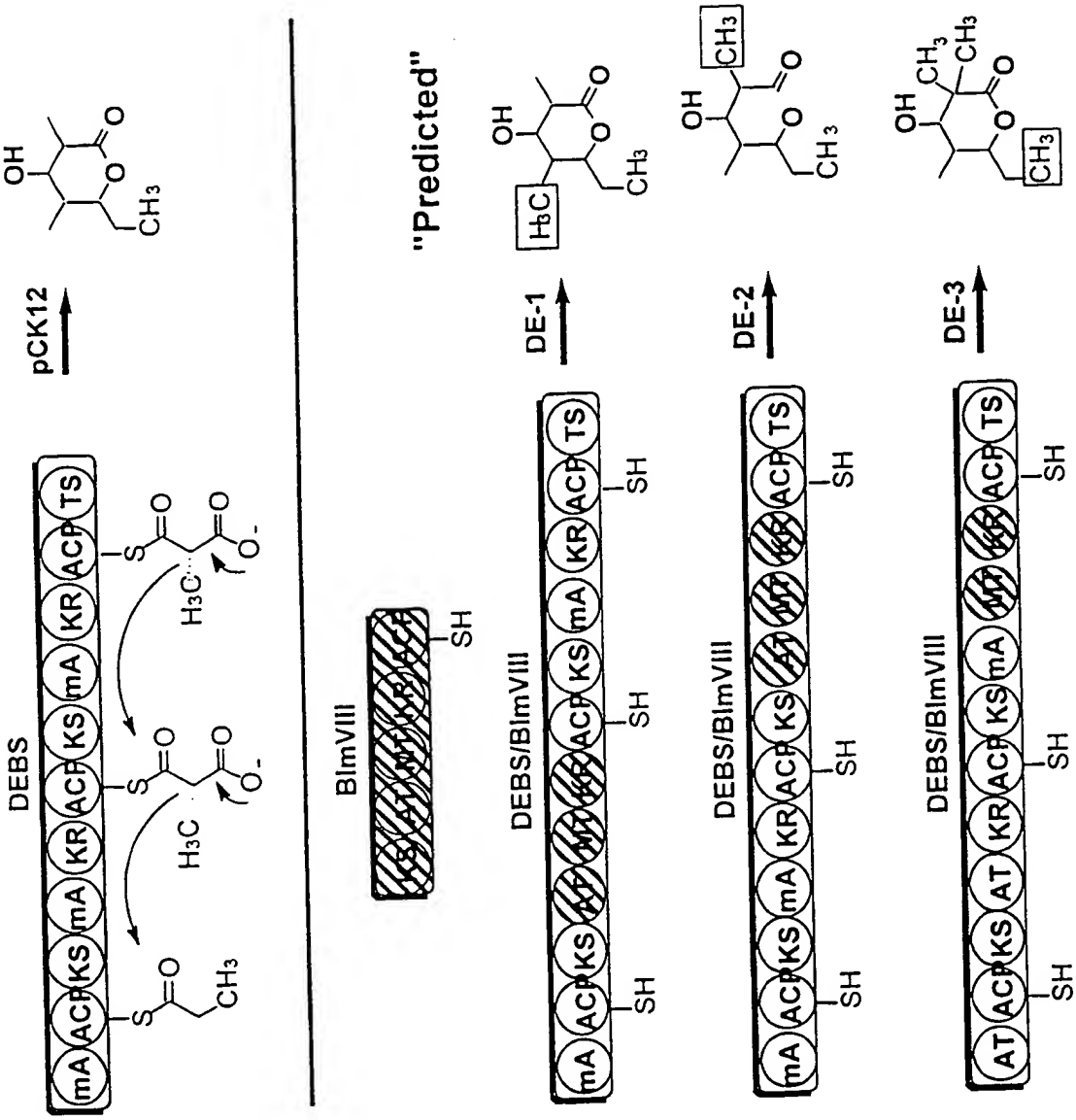

DEBS
mA
ACP
KS
mA
KR
ACP
TS
pCK12
OH
CH3
H3C
BlmVIII
SH
"Predicted"
DE-1
DE-2
DE-3
DEBS/BlmVIII
AT
KS
ACP
KR
TS

FIG. 5

FIG. 6A

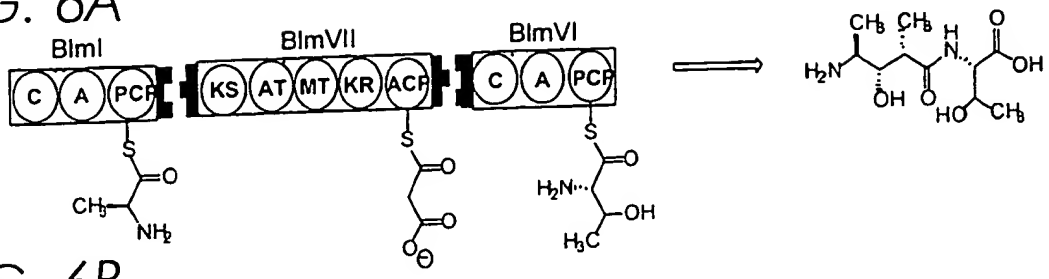


FIG. 6B

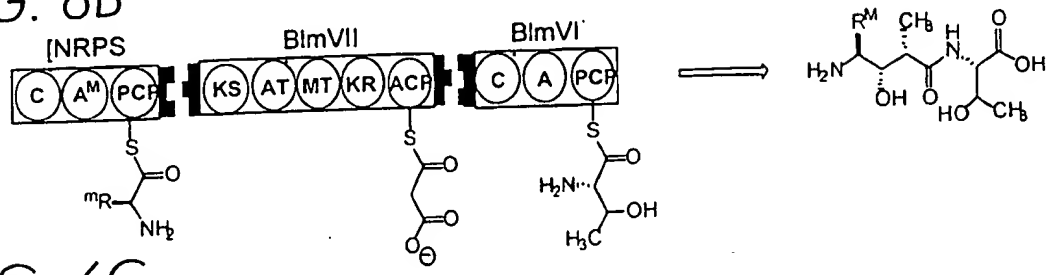


FIG. 6C

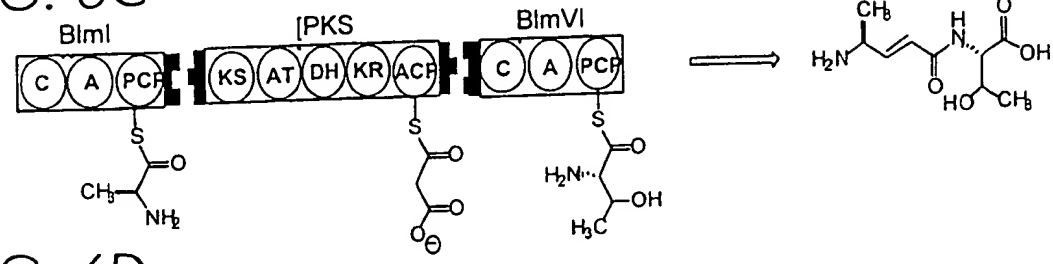


FIG. 6D

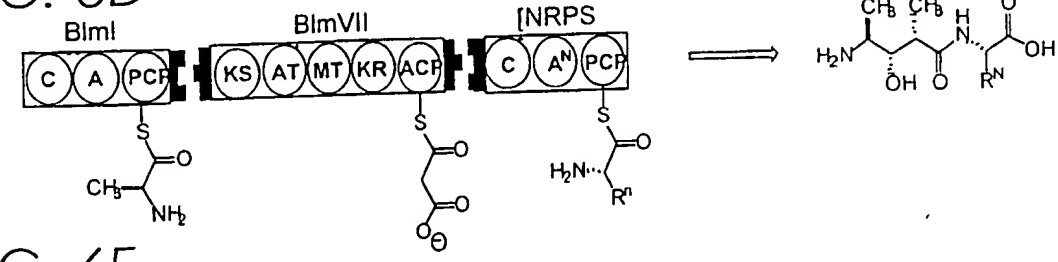


FIG. 6E

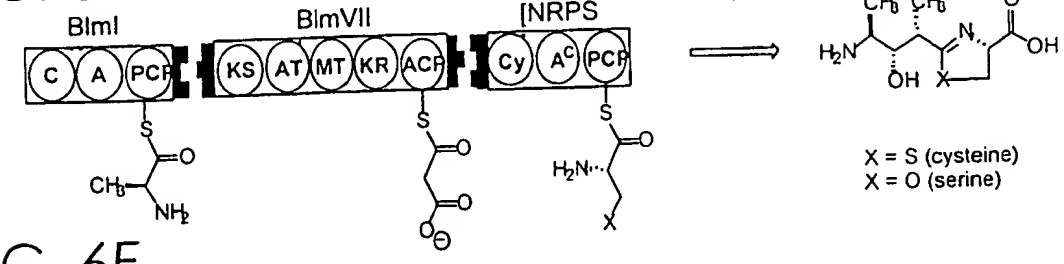
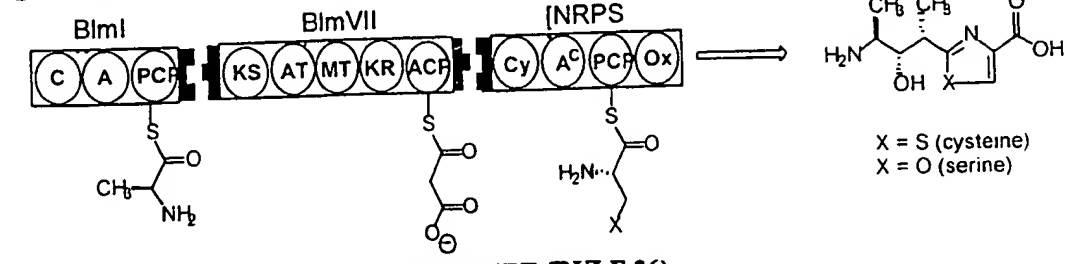


FIG. 6F



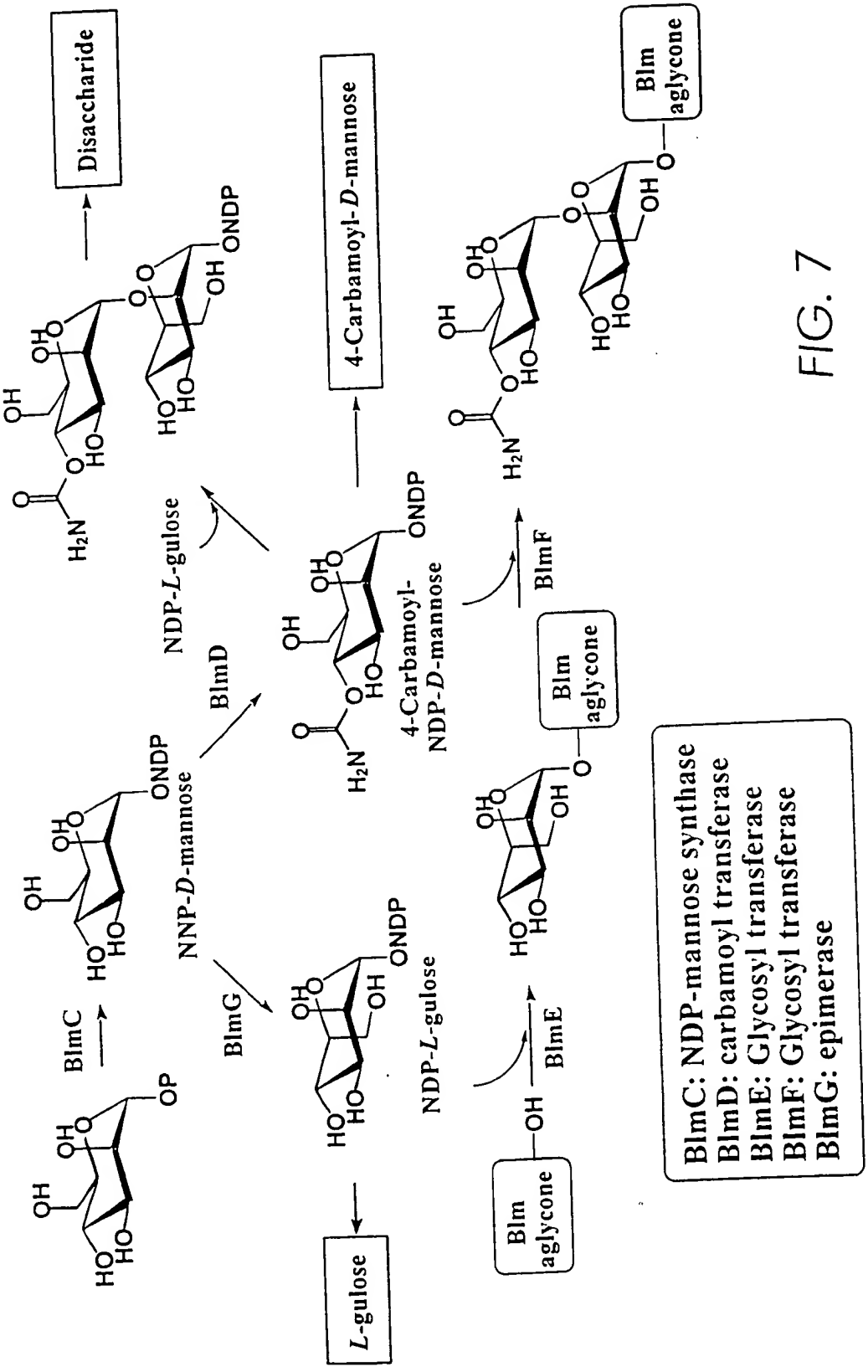
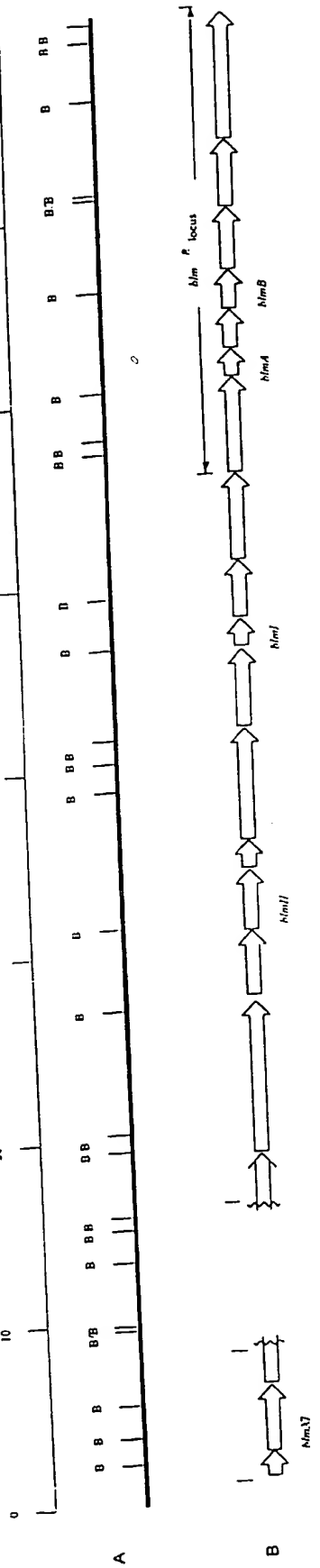

Disaccharide
4-Carbamoyl-D-mannose
Blm aglycone
NDP-L-gulose
BlmD
BlmF
BlmC
BlmG
BlmE
NNP-D-mannose
4-Carbamoyl-NDP-D-mannose
NDP-L-gulose
L-gulose
Blm aglycone
Blm aglycone
BlmC: NDP-mannose synthase
BlmD: carbamoyl transferase
BlmE: Glycosyl transferase
BlmF: Glycosyl transferase
BlmG: epimerase

FIG. 7

FIG. 8


0
10
20
30
40
50
60
70
80 kb
A
B
C
hlm R locus
hlmA
hlmB
hlmI
hlmII
hlmM
-18 RBS
CCGGACGGCGGCCCGCTC
ATGAGCGCCCCCGCGGGGCGAGCGGACCCGGCGCCGCGCTCGAACGCGACATCGCCGCGATCTGGGCCGAGACCCTCGGCAGGGACAGCGTC 93
M S A P R G E R T R R R A L E R D I A A I W A E T L G R D S V 186
GGCCCGCACGAGGACTTCGCCGCGCTGGGCGGCAACTCCATCCACGCCATCAAGATCACCAACCGGGTGGAGGAACTCGTCGACGCCGAGCTG
G P H E D F A A L G G G N S I H A I K I T N R V E E L V D A E L 273
TCCATCCGCGTCCTGCTCGAGACGGCACCGTGGCCGGCATGACGGACCACGTCCACGCCACGCTCACGGGGGAGCGGGACCGGTGA
S I R V L L E T R T V A G M T D H V H A T L T G E R D R *

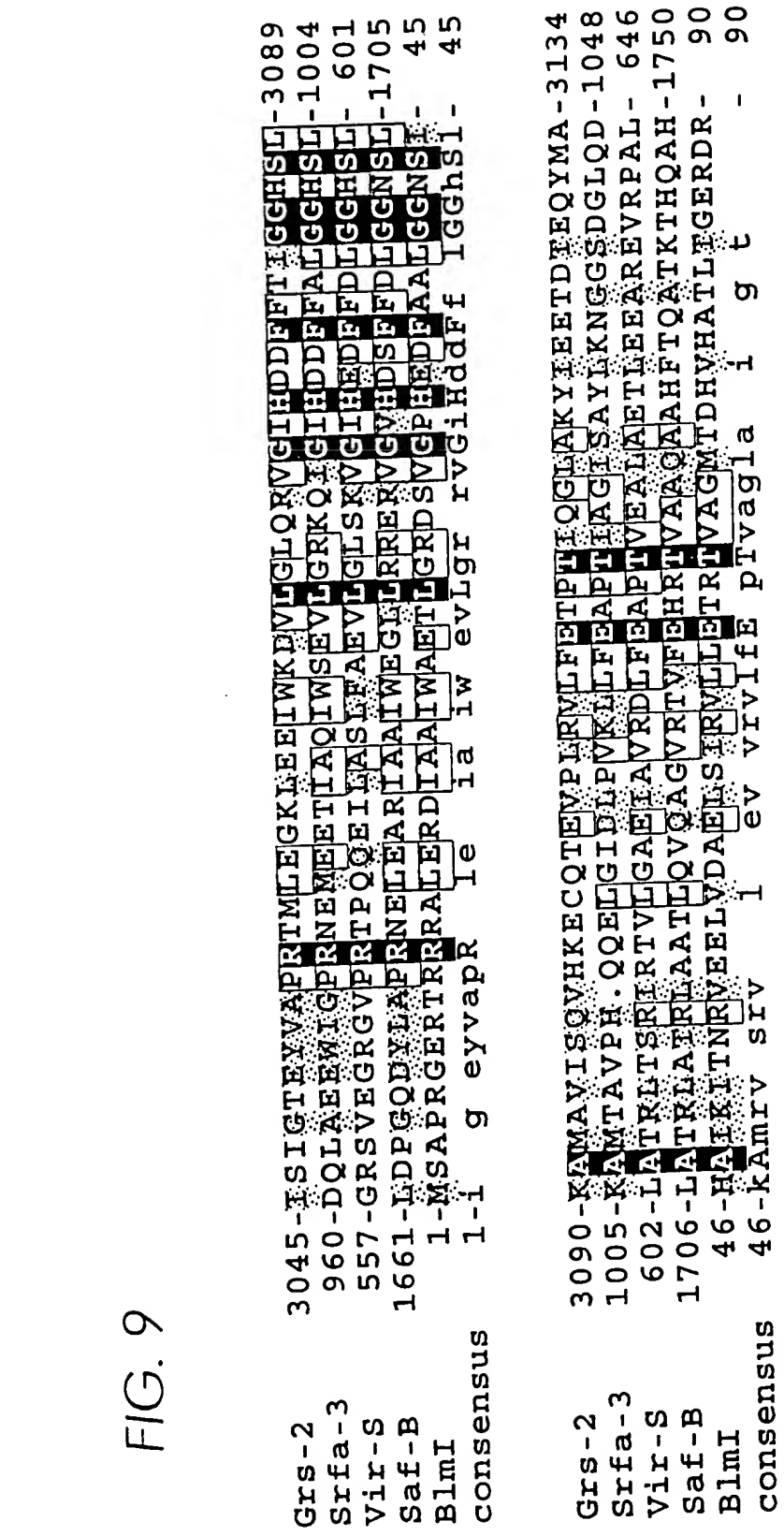

Grs-2 3045-ISIGTEYVAPRTMLEGKLEEIWKDVLGLQRVGIHDDFFTIGGHSL-3089
Srfa-3 960-DQLAEEWIGPRNEMEETIAQIWSEVLGRKQIGIHDDFFALGGHSL-1004
Vir-S 557-GRSVEGRGVPRTPQQEILASLFAEVLGLSKVGIHEDFFDLGGHSL- 601
Saf-B 1661-LDPGQDYIAPRNELEARIAAIWEGLLRRERVGVHDSFFDLGGNSL-1705
BlmI 1-MSAPRGERTRRALERDIAAIWAETLGRDSVGPHEDFAALGGNSI- 45
consensus 1-i g eyvapR le ia iw evLgr rvGiHddFf IGGhSl- 45
Grs-2 3090-KAMAVISQVHKECQTEVPIRVLFETPTIQGLAKYIEETDTEQYMA-3134
Srfa-3 1005-KAMTAVPH.QQELGIDIPVKLLFEAPTIAGISAYIKNGGSDGLQD-1048
Vir-S 602-LATRITSRERTVLGAEIAVRDLFEAPTVEALAETLEEAREVRPAL- 646
Saf-B 1706-LATRIAIRLAATLQVQAGVRTVFEHRTVAAQAAHFTQATKTHQAH-1750
BlmI 46-HAIKITNRVEELVDAELSIRVLETRTVAGMTDHVHATLTGERDR- 90
consensus 46-kAmrv srv i ev vrvlfE pTvagla i g t - 90

FIG. 9

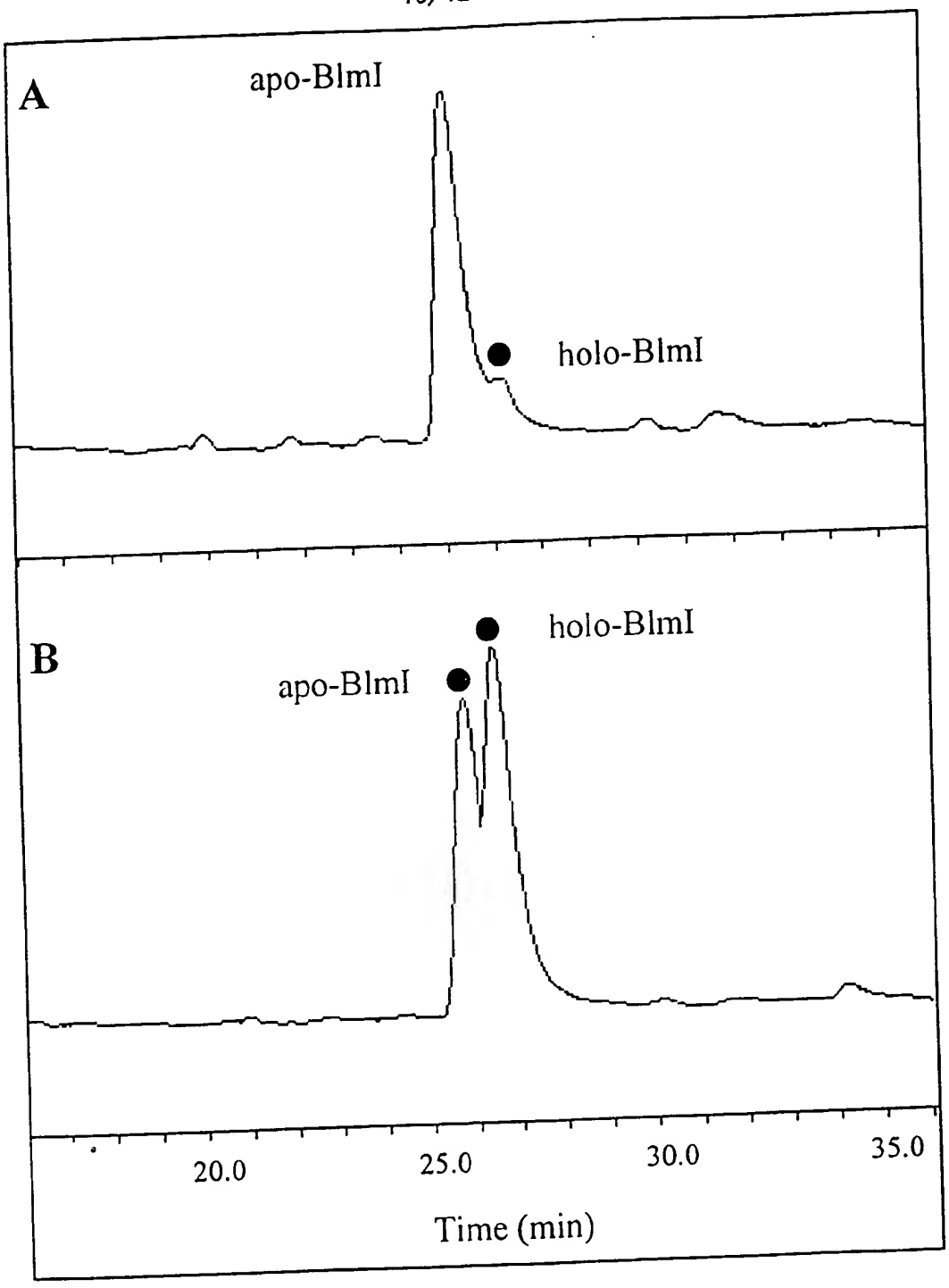

A
apo-BlmI
holo-BlmI
B
holo-BlmI
apo-BlmI
20.0
25.0
30.0
35.0
Time (min)

FIG. 10

Nonreiterative Type I Modular Protein Template

FIG. 11A

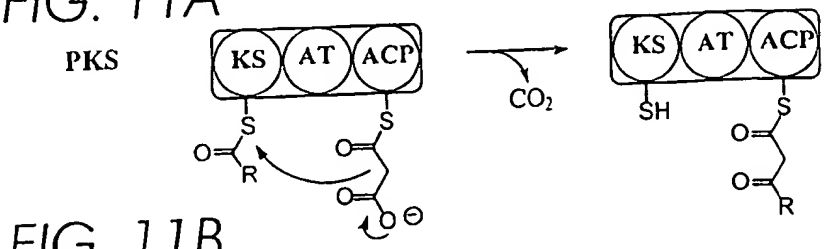
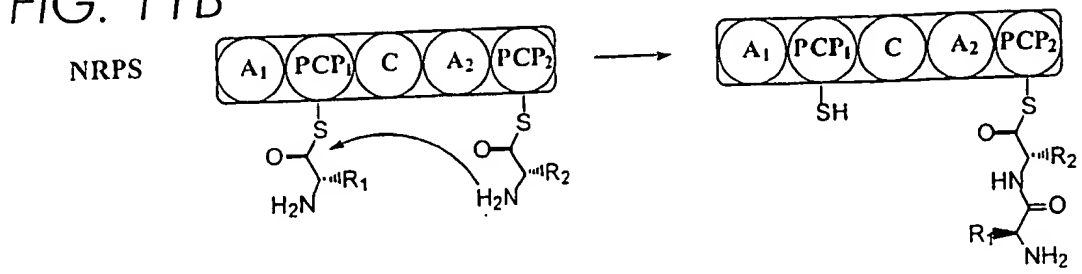


FIG. 11B



Iterative Type II Protein Complex

FIG. 11C

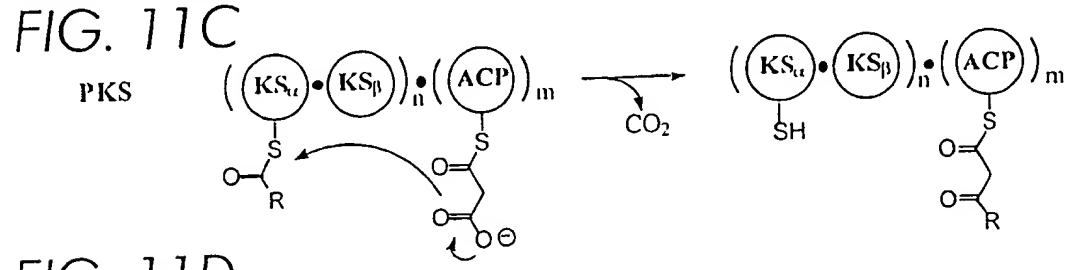


FIG. 11D

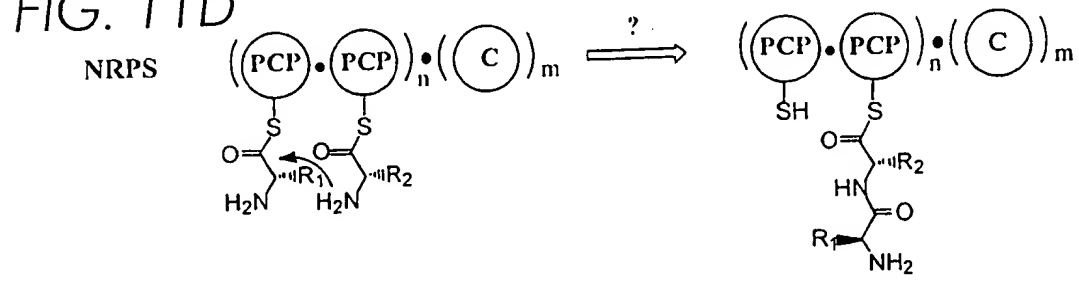


FIG. 12

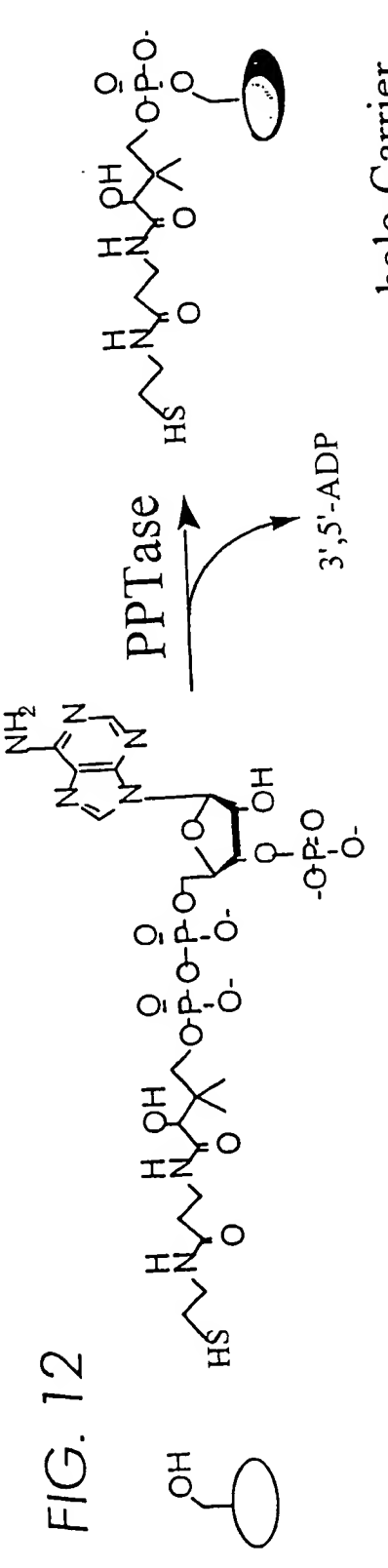
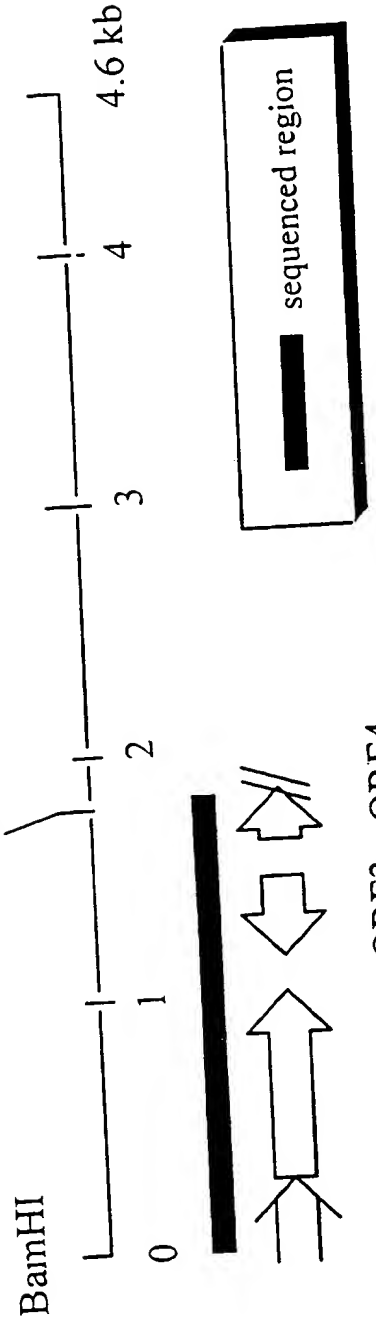

PPTase
3',5'-ADP
holo-Carrier Protein
Coenzyme A
apo-Carrier Protein

FIG. 13


BamHI
SalI
BamHI
0
1
2
3
4
4.6 kb
sequenced region
ORF1
pptA
PPTase
ORF3
ORF4

SEQUENCE LISTING

SEQ ID NO: 1 BLM gene cluster ORFS 30 through 8

(note orf 31-40 on sequence 1-18660 are translated on the reverse strand and on a separate file)

```
18601 ACCCATCTCATAGGTGTACGCGCTGGAGCATTCGGGGCACGACGGAAGGTTCTCGGTCAC 18660
18661 GAGAGCACTGTAAGCCCGAACCCGCAAGGATGACGAATTGCAAAATTGTGCAAGTCGCTA 18720
18721 CATGATGGTCCGGCTGTGCCCGCAGGTAGCCGCGGGCACAGCACCAGACGCTGCCTCCGC 18780
18781 GCACCGCGCGGGAGGCCCGGTGAGGCGAGAGGCTGAGGTTCCGTGCCGGTTCCGCTGTAT 18840
                                                  M  P  V  P  L  Y  (orf30)

18841 CAGGCGAAGGCCGAGTTCTTCCGGATGCTGGGGCACCCGGTCCGCATCCGCGTACTGGAG 18900
      Q  A  K  A  E  F  F  R  M  L  G  H  P  V  R  I  R  V  L  E

18901 CTGCTGCAGGACGGGCCGATGCCGGTGCGTGATCTGCTGGCGGCGATCGAGATCGAGCCC 18960
      L  L  Q  D  G  P  M  P  V  R  D  L  L  A  A  I  E  I  E  P

18961 TCGGCGCTGTCCCAGCAGCTGGCGGTGTTGCGCCGCTCGGGCATCGTGACCTCCACCCGC 19020
      S  A  L  S  Q  Q  L  A  V  L  R  R  S  G  I  V  T  S  T  R

19021 ACGGGTTCCACGGTCGTCTACGAGCTGGCCGGTGGCGACGTGGCGGAGCTGATGTCCGCC 19080
      T  G  S  T  V  V  Y  E  L  A  G  G  D  V  A  E  L  M  S  A

19081 GCGCGCCGCATCCTGACCGAGATGCTCAATGGGCAGCACGAGCTGCTGGAGGAGCTGAGG 19140
      A  R  R  I  L  T  E  M  L  N  G  Q  H  E  L  L  E  E  L  R

19141 GAAGCCGAGGTCAGTGCCCGGTGAGCTCCCTCGCCGTCCGGGTGGGAGCCCGGGTGCGTT 19200
      E  A  E  V  S  A  R  *
                        M  S  S  L  A  V  R  V  G  A  R  V  R  S  (orf29)

19201 CCGTGCTGCCCACCCGCGCCGACCTCGCGGGCATGGGCCGCAGCCCGCGACGTGATCTAC 19260
        V  L  P  T  R  A  D  L  A  G  M  G  R  S  P  R  R  D  L  L

19261 TGGCCGGTCTGACCGTGGCGATCGTGGCCCTGCCGCTCGCCCTCGGATTCGGCGTCTCCT 19320
        A  G  L  T  V  A  I  V  A  L  P  L  A  L  G  F  G  V  S  S

19321 CCGGTCTCGGCGCGGAGGCAGGGCTGGCCACCGCGGTGGTGGCGGGCGCGCTGGCCGCGG 19380
        G  L  G  A  E  A  G  L  A  T  A  V  V  A  G  A  L  A  A  V

19381 TATTCGGTGGGTCGAATCTCCAGGTGTCCGGGCCCACGGGCGCCATGACCGTGGTCCTGG 19440
        F  G  G  S  N  L  Q  V  S  G  P  T  G  A  M  T  V  V  L  V

19441 TGCCCATCGTCGCCCGGTACGGCCCCGGCGGTGTCCTCACGGTCGGCCTGCTCGCCGGAC 19500
        P  I  V  A  R  Y  G  P  G  G  V  L  T  V  G  L  L  A  G  L

19501 TGATGCTGATCGCGCTCGCCCTCGCCCGCGCCGGCGCTACATGCAGTACGTGCCGGCCC 19560
        M  L  I  A  L  A  L  A  R  A  G  R  Y  M  Q  Y  V  P  A  P

19561 CGGTGGTGGAGGGCTTCACCCTCGGCATCGCCTGCGTGATCGGCTTGCAGCAGGTGCCGA 19620
        V  V  E  G  F  T  L  G  I  A  C  V  I  G  L  Q  Q  V  P  N

19621 ACGCCCTGGGAGTCGCCAAGCCGGAGGGCGACAAGGTCCTCGTCGTGACCTGGCGCGCGG 19680
        A  L  G  V  A  K  P  E  G  D  K  V  L  V  V  T  W  R  A  V

19681 TCGAGACCTTCGCCGGGGCGCCCAACTGGACCGCTGCCGGACTGGCGGCAGCGGTCGCCG 19740
        E  T  F  A  G  A  P  N  W  T  A  A  G  L  A  A  A  V  A  A

19741 CGGTCATGCTGACCGGCGCGCGGTGGCGGCCGGTCGTTCCCTTCTCCCTCCTCGCGGTGA 19800
        V  M  L  T  G  A  R  W  R  P  V  V  P  F  S  L  L  A  V  T

19801 CCGGTGCCACCGTCGTGGCCCAGCTGTGCCACCTGGACGCGGCCCGCCCGATCGGGGACC 19860
        G  A  T  V  V  A  Q  L  C  H  L  D  A  A  R  P  I  G  D  L

19861 TGCCCGCGGGGCTGCCCGCCCCGTCGCTGGCCTTCCTGGACCTCGGAGCACTGGGCTCGC 19920
        P  A  G  L  P  A  P  S  L  A  F  L  D  L  G  A  L  G  S  L

19921 TGCTGGCGCCTGCCGTGGCCGTGGCGGCCCTTGCCGCGTTGGAATCGCTGCTGTCGGCGT 19980
        L  A  P  A  V  A  V  A  A  L  A  A  L  E  S  L  L  S  A  S
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19981 CCGTCGCGGACGGCATGACGGTCGGGCAGAAGCACGACCCGGACAAGGAGCTGTTCGGGC 20040
        V  A  D  G  M  T  V  G  Q  K  H  D  P  D  K  E  L  F  G  Q
20041 AGGGTCTCGCCAACCTGGCCGCCCCGCTGTTCGGCGGCGTCCCGGCCACCGGCGCGATAG 20100
        G  L  A  N  L  A  A  P  L  F  G  G  V  P  A  T  G  A  I  A
20101 CCCGCACCGCCGTCAACGTCCGTACCGGTGCGAGCTCGCGACTGGCGGCCCTCACGCACG 20160
        R  T  A  V  N  V  R  T  G  A  S  S  R  L  A  A  L  T  H  A
20161 CCGCGATCCTCGCCGTCATCGTCTTCGCCGCCGCCCCACTGGTCTCCCGCATCCCCCTGG 20220
        A  I  L  A  V  I  V  F  A  A  A  P  L  V  S  R  I  P  L  A
20221 CCGCGCTCGCCGGCGTGCTGATCGCGACCGCGATCCGCATGGTCGAAGTGGGCAGCCTGC 20280
        A  L  A  G  V  L  I  A  T  A  I  R  M  V  E  V  G  S  L  R
20281 GGGCGATGGCCCGCGCCACGCGCTCCGACGGCCTGGTACTGATCCTCACGGCGGTCGCCA 20340
        A  M  A  R  A  T  R  S  D  G  L  V  L  I  L  T  A  V  A  T
20341 CCGTGGCCCTGGACCTCGTCTACGCCGTCATCATCGGCCTGCTGGTCGCCGGCGCACTCG 20400
        V  A  L  D  L  V  Y  A  V  I  I  G  L  L  V  A  G  A  L  A
20401 CCCTGCGGGCCGTGGCCAAGCAGGTCCGCCTGGACCAGGTCTCCTTGAAGGAGGACCTGA 20460
        L  R  A  V  A  K  Q  V  R  L  D  Q  V  S  L  K  E  D  L  T
20461 CCGGCGACCACAGCGCCGAGGAACACGCGCTGCTCGCCGAGCACATCGTGGCGTACCGCA 20520
        G  D  H  S  A  E  E  H  A  L  L  A  E  H  I  V  A  Y  R  I
20521 TCGACGGTCCGCTGTTCTTCGCCGCGGCCCACCGCTTCCTGCTGGAACTCTCGGACGTCG 20580
        D  G  P  L  F  F  A  A  A  H  R  F  L  L  E  L  S  D  V  A
20581 CGGACGTGCGCGTGGTGATCCTGCGCATGTCCCGCGTGACCACCATGGACGCCACCGGCG 20640
        D  V  R  V  V  I  L  R  M  S  R  V  T  T  M  D  A  T  G  A
20641 CCCTCGTCCTGAAGGACGCGGTCACCAAGCTGAACCGGCGCGGCATCACCGTCCTGGCCT 20700
        L  V  L  K  D  A  V  T  K  L  N  R  R  G  I  T  V  L  A  S
20701 CCGGGGTACGCCCCGGCCAGCGCCGGGTCCTCGACTCCGTCGGCGCCCTCGGTCTGCTCC 20760
        G  V  R  P  G  Q  R  R  V  L  D  S  V  G  A  L  G  L  L  R
20761 GGGCCGCCACCGGCGACGACTACACCGGCACTCCCGAAGCCATCGCCGCCGCCCGAAGCC 20820
        A  A  T  G  D  D  Y  T  G  T  P  E  A  I  A  A  A  R  S  H
20821 ACCTGCACGGCGCCGGTGTCCTGGCCCCCGCCTGCCCGGGCCCGCCTCCTCCGGTACCCC 20880
        L  H  G  A  G  V  L  A  P  A  C  P  G  P  P  P  P  V  P  P
20881 CACCGTGCGCTCCGAGTGCCCGACGATGAGGAGCCGACCGAGGTCCTCCTCCGTCACCCG 20940
        P  C  A  P  S  A  R  R  *
20941 GACACCCACGGTTGCGCCGCCCCATGCCGGCGGTCCCTCCTGACGGCCCGTCCGCGGCTT 21000
21001 GAGGCGGCGGTGGACGGCCTGCCGCCGCCGGCCTCGGGCTGATCGGCGTGATCACCGCCC 21060
21061 ATGCGCGGGTGGGCGCCCGCGGCATCGTGGGCGGGACCGTGTTCCCGGCCACCGCGGCGG 21120
21121 CCGGCCTCGCGCTGGGCGTGGCCTGCCGCGGTGCCTGGTAGCGGCGGGGTCCGGCGGCCG 21180
21181 GGCCTGTGCTTCTTCCCGCCCGTCCGGCGGGTGGCGCCGCGCCGGCGGTGACAGGGAAAT 21240
21241 ATGACCGGAACTGGGATGCTCGCGTCCACTCGGGTGTGTTTAAGTGCCACGGGGGCTTCC 21300
21301 GACGGCGCGTCGCGCGCCGGCGGTTCGCCCGATGATGGTCGTGCGGCGCTGTGAGCCGGG 21360
21361 GAGCCTATGGCACAGGACCTGAACGACTGGATCGAGGACGAGGTCGTCCCTTACGAGGAG 21420
                M  A  Q  D  L  N  D  W  I  E  D  E  V  V  P  Y  E  E   (orf28)
21421 AAGCCTCTCGAATGGATCTCCCAGTACCACTTCTTCCGCGACCCGGCGCGAGCCGCCTAT 21480
        K  P  L  E  W  I  S  Q  Y  H  F  F  R  D  P  A  R  A  A  Y
21481 GTCGATCACACCTACTTCTTCTCACCGGCCGATGGCGCGATCGTCTACCAGAAAGTAGTG 21540
        V  D  H  T  Y  F  F  S  P  A  D  G  A  I  V  Y  Q  K  V  V
21541 GATCCCCAGGAGTCGATCATCGACATCAAGGGGAAGCCGTACTCGCTGGCCGCCGCGCTC 21600
        D  P  Q  E  S  I  I  D  I  K  G  K  P  Y  S  L  A  A  A  L
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21601 CGTGACGAATCGTTCGGTCACCGGTGCCTGGTGATCGGCATCTTCATGACCTTCTTCGAC 21660
      R  D  E  S  F  G  H  R  C  L  V  I  G  I  F  M  T  F  F  D

21661 GTGCACATCAACCGGATGCCTTACGGCGGCCGTCTCTCCTTCGCGCTCAAGGAGCCCATC 21720
      V  H  I  N  R  M  P  Y  G  G  R  L  S  F  A  L  K  E  P  I

21721 GGGACGTTCAACCTCCCCATGCTGGCCATGGAGCAGGACCTGCTCGAACGGCTCCGGGTC 21780
      G  T  F  N  L  P  M  L  A  M  E  Q  D  L  L  E  R  L  R  V

21781 AATCCGGCTCACGCGAGGTATCTGCACCTGAACGAGCGGATGGTCAACCGGGTCGACGCG 21840
      N  P  A  H  A  R  Y  L  H  L  N  E  R  M  V  N  R  V  D  A

21841 CCGCGGCTCCGGGGCCCGTACTGGATGCTCCAGATCGCCGACTACGACGTCGACTCCATC 21900
      P  R  L  R  G  P  Y  W  M  L  Q  I  A  D  Y  D  V  D  S  I

21901 ACCCCGTTCTGCAGACGGCAGGGAATGTTCCGCTCCCAGGGGCGCCGCTTCTCCCAGATC 21960
      T  P  F  C  R  R  Q  G  M  F  R  S  Q  G  R  R  F  S  Q  I

21961 CGCTACGGATCGCAGGTCGACCTGGTGATCCCGATGGCGGCCGACCGCGAGTACGTCCCC 22020
      R  Y  G  S  Q  V  D  L  V  I  P  M  A  A  D  R  E  Y  V  P

22021 GTGGAGGCCGTCGGCCGGCACGTGAAGGCGGGGCTCGACCCGCTCGTCAAGATCCGGTGG 22080
      V  E  A  V  G  R  H  V  K  A  G  L  D  P  L  V  K  I  R  W

22081 CGTTGAAGAGCGCGTACGAAGCGATGGCGAACTGGAGGGACACAGCGTGGGTTTCCGTCG 22140
      R  *                                                M  G  F  R  R  (orf27)

22141 AGCGCAGAGGGCCGGTGGGCCGGGAGCGGGCCGGCGGGAGAGCGCCCGGTTCAGGCCGGA 22200
      A  Q  R  A  G  G  P  G  A  G  R  R  E  S  A  R  F  R  P  D

22201 CGGGCCGTCGGCGCCGCGGGACCGTCCGTTACCCCTGTCCGCCGGGCAGTTGTTCGAGTG 22260
      G  P  S  A  P  R  D  R  P  L  P  L  S  A  G  Q  L  F  E  W

22261 GGTGTTTGACAAGCTCGTCGACGGAGATCTGAGCCACCAGCCGACGATTGTGCGGCTCCG 22320
      V  F  D  K  L  V  D  G  D  L  S  H  Q  P  T  I  V  R  L  R

22321 CGGCCCGCTGAACACCGCCGCCCTGCGGATGGCCTACGCCCGGCTGGTGCGGCGCCACGA 22380
      G  P  L  N  T  A  A  L  R  M  A  Y  A  R  L  V  R  R  H  E

22381 GTGCCTGCGCACCCGCTTCCCCGTGATCGACGGGGAGCCCGTGCAGGTGATCGAGGGCAT 22440
      C  L  R  T  R  F  P  V  I  D  G  E  P  V  Q  V  I  E  G  I

22441 CGGGAAAGCAGCGGGGGGCCCGCTGCCGCTCATCGATCTGCGCCACCTCCCGGAGGCGCT 22500
      G  K  A  A  G  G  P  L  P  L  I  D  L  R  H  L  P  E  A  L

22501 TCGCGCGCGCGAGATCGCGAGGATCCGCGAGGAGACGCTGTCCACGCCGGTCCCCTTCGA 22560
      R  A  R  E  I  A  R  I  R  E  E  T  L  S  T  P  V  P  F  D

22561 CAAGCGGCCGCCCGTCCGCGTGGCGCTGATCCGGGCGGCGCCCGAGGAGCACCTCTTCCT 22620
      K  R  P  P  V  R  V  A  L  I  R  A  A  P  E  E  H  L  F  L

22621 CGTCGGCATCCCGCACATCACCGCGGACCTGTGGTCCGCGACCCTGCTCAACGACGAGCT 22680
      V  G  I  P  H  I  T  A  D  L  W  S  A  T  L  L  N  D  E  L

22681 CATGGCGCACTACAGGGCGGGGGCCGAGGGGACTCCCTCCCGGGCCCCCACCCCCGTCGC 22740
      M  A  H  Y  R  A  G  A  E  G  T  P  S  R  A  P  T  P  V  A

22741 GCAGTACGCCGACTTCGCGCAGTGGCAGCGCGCGTGGTGGAACCGGGACCGCACCGAGCG 22800
      Q  Y  A  D  F  A  Q  W  Q  R  A  W  W  N  R  D  R  T  E  R

22801 GGAGGCCGGACGGTGGCGGGCGCGGCTGGACGGGCTGTCCGCCGTGGAACTGCCCCTGGA 22860
      E  A  G  R  W  R  A  R  L  D  G  L  S  A  V  E  L  P  L  D

22861 CCGGCCCCGCCCCGCGGGCCGCCGGCGGGACTGCTTCCTGATCGGGGACACCTTCGACGC 22920
      R  P  R  P  A  G  R  R  R  D  C  F  L  I  G  D  T  F  D  A

22921 CGAACTGAGCGACCGGCTGCGCGCCTTGGCACGCACCGCCGACGTCACGCTGTACGTGGT 22980
      E  L  S  D  R  L  R  A  L  A  R  T  A  D  V  T  L  Y  V  V

22981 GCTGCTGGCGGCGTTCCACTGGCTGGTGGGGCGGATGTCGGGCGCCGGCCGGCTGGTGAC 23040
      L  L  A  A  F  H  W  L  V  G  R  M  S  G  A  G  R  L  V  T

23041 CACCTCGCTCGTGGCCGCCCGGCACGGCAGCGCGGTACAGGGGATGACCGGCCCGTTCTC 23100
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          T  S  L  V  A  A  R  H  G  S  A  V  Q  G  M  T  G  P  F  S

23101 GGACTACCTGGCCCTGGTCGGGGACCTGTCGGGCGATCCGGACTTCCTGGAGTCCCTGCG 23160
          D  Y  L  A  L  V  G  D  L  S  G  D  P  D  F  L  E  S  L  R

23161 CCGCGTACGCGACGAGTGCCTGACCGCCCACGACCACCAGCGGCTTCCGTTCTCACAGGT 23220
          R  V  R  D  E  C  L  T  A  H  D  H  Q  R  L  P  F  S  Q  V

23221 CCTCGAAGTCATGGACCCCGGACGCGAGTTGCACCCCCATCCGCTGGAGCAGCTCGGGTT 23280
          L  E  V  M  D  P  G  R  E  L  H  P  H  P  L  E  Q  L  G  F

23281 CAACCTCCACAACATCCCTCCCGCGGTCATGGACTTCTCCGGCGACGTCGTCGTCTCGGC 23340
          N  L  H  N  I  P  P  A  V  M  D  F  S  G  D  V  V  V  S  A

23341 GGTGAACCCGGAGGGGGACGACGGGGAGAGCGGCGACGGGGAGTACGTGCCCTGGACCGC 23400
          V  N  P  E  G  D  D  G  E  S  G  D  G  E  Y  V  P  W  T  A

23401 CGACCTGACCTTCGACGTCTACGACTACGGCACCGGCCATATGCCGTTCGACGTGATACT 23460
          D  L  T  F  D  V  Y  D  Y  G  T  G  H  M  P  F  D  V  I  L

23461 CGACCGGCGGCTGGCCGATCCGGCGACGGCCCGGGAGTGGGCCGGGCACTACCGGTCGGT 23520
          D  R  R  L  A  D  P  A  T  A  R  E  W  A  G  H  Y  R  S  V

23521 GCTCCGTGCGGTCGTCGCCGACCCCGGCGTGCGCCTGTCCGCCCTCGGCACCCTGCTGTC 23580
          L  R  A  V  V  A  D  P  G  V  R  L  S  A  L  G  T  L  L  S

23581 CCTGCCGCGACCGCCGTCCGCCACGTCCTTCGGCGGCCGGGAGATCGACGTCCGGCGCGT 23640
          L  P  R  P  P  S  A  T  S  F  G  G  R  E  I  D  V  R  R  V

23641 CGAACGCGAGTTGGCGGGGCGCGACGGGATCACCGCCGCCCTGGTCGCGGTGGCGCCCCG 23700
          E  R  E  L  A  G  R  D  G  I  T  A  A  L  V  A  V  A  P  R

23701 GCGCCTGGCCACCGGGCTGCGCGTACGGGAACTGGTCGCCTACTGCGCCGTCGAGGGCAC 23760
          R  L  A  T  G  L  R  V  R  E  L  V  A  Y  C  A  V  E  G  T

23761 GCCGCGTCCGAACGCGGCCCACGACATCCGCGGCCGCCTGCGGGAGCGCCTGCCCGACGG 23820
          P  R  P  N  A  A  H  D  I  R  G  R  L  R  E  R  L  P  D  G

23821 CTGGGTGCCGACCGTGTTCGTCGAGCGCCCGCCGGAGGAGATCCGGAAGGCCCTGGCCGC 23880
          W  V  P  T  V  F  V  E  R  P  P  E  E  I  R  K  A  L  A  A

23881 CCGGGCGGCGGGCGGCGAACGGGCGGAGCCGCTGCCGCCGCCCGAGGACTGCGTCCCGCT 23940
          R  A  A  G  G  E  R  A  E  P  L  P  P  P  E  D  C  V  P  L

23941 TCCCGAGGAGGGCCGGCCCCCCTCGGACCCGTCCGAGCGGCGGCTGGCCGCGCTCTGGGC 24000
          P  E  E  G  R  P  P  S  D  P  S  E  R  R  L  A  A  L  W  A

24001 CGAGATCCTGGGCGCCCCGCCGAAGAGCGTGACCGAGCCCTTCTTCCGCGTCGGCGTCAC 24060
          E  I  L  G  A  P  P  K  S  V  T  E  P  F  F  R  V  G  V  T

24061 CGATAAGGACGCCCTCCGCTTCCTGGCCCGCGTGGCGGAGGACTTCGGCGTCACCGTGCC 24120
          D  K  D  A  L  R  F  L  A  R  V  A  E  D  F  G  V  T  V  P

24121 CTTCGCCGACTTCCTCAGCGCTCCCAACCTGCGTATGGTGAAGGACAATTTGGCTGAGAA 24180
          F  A  D  F  L  S  A  P  N  L  R  M  V  K  D  N  L  A  E  K

24181 ACGGAGGGTGTAACGCGCAATGAGTGAGTGGTAGGGTCGGAATCGAACCGCACTGATCGG 24240
          R  R  V  *

24241 CAATCTTTTCGGTCAGCTGTTCCGGATATTCCGGGGCGCGTCGGCGCTCCCTCGACCAAG 24300

24301 GGCGTACGCGGATAAGCGTGCGCCGCCCCACGGCTGCGTCTCGACGCCTTCATCGGCGCG 24360

24361 TCGGACACTTCGCGGTGCCAGTCGGCACGCTCAGAGATCAGTGGAATGCCTCGGTGTGCC 24420
                                                    M  P  R  C  A  (orf26)

24421 CGAGGTGCGCTCAGTACTGCTGTCCACACAACGCGCCAAGGGAGTTGGAACGTGATGGAG 24480
          R  G  A  L  S  T  A  V  H  T  T  R  Q  G  S  W  N  V  M  E

24481 ACGGCGAATTCCGGCTATCGGGTCTCACCTCAGCAGCGGCATTTATGGGCCATGCTGACC 24540
          T  A  N  S  G  Y  R  V  S  P  Q  Q  R  H  L  W  A  M  L  T

24541 CGCGGGCGGGACGGCGGCGACGTGCGTTCACCCAGTCCGCCGTGGTGGTCGACCGTTCC 24600
          R  G  R  D  G  G  R  R  A  F  T  Q  S  A  V  V  V  D  R  S
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24601 CTGGACGCCGCACGTCTGCGCGCCGCGCTGGCCTCCGTGGTGGCCGCCCACGAGCCGCTG 24660
      L  D  A  A  R  L  R  A  A  L  A  S  V  V  A  A  H  E  P  L

24661 CGGACGACCTTCACCGGTCTCGCGGGACGGACCGCGCCGGTCCAGGTCGTCCATGACCCG 24720
      R  T  T  F  T  G  L  A  G  R  T  A  P  V  Q  V  V  H  D  P

24721 GACGAGCAGCCGCTGTCCGTCGTCGACCTGCCGCCCTCGTGCGCCGACGGCTCGGGCCCG 24780
      D  E  Q  P  L  S  V  V  D  L  P  P  S  C  A  D  G  S  G  P

24781 GAACTGGACGAGCTCCGGCTCCGCGAACGCGCCGCCCTCGACCCGCGCGGCGGGCCCGTC 24840
      E  L  D  E  L  R  L  R  E  R  A  A  L  D  P  R  G  G  P  V

24841 TTCCGGGCCGCCCTGGCGCGGGCCGGCGAGGACCGGGCGGTGCTGGTGCTCACCGCGCAC 24900
      F  R  A  A  L  A  R  A  G  E  D  R  A  V  L  V  L  T  A  H

24901 GCCCTGGTCGCGGACCGGCTCTCCCTCCGGCTGCTGGCCGGGCAGATCCTCGCGGCGTAC 24960
      A  L  V  A  D  R  L  S  L  R  L  L  A  G  Q  I  L  A  A  Y

24961 AGCGGGGAGACCGTGTCCCCCGATGGCCCGCCGCCCTTGCAGTACGCCGACTTCGCCGCC 25020
      S  G  E  T  V  S  P  D  G  P  P  P  L  Q  Y  A  D  F  A  A

25021 TGGCACCACGACCTGCTCACCGCCGAGGACGCCGCCCCCGACCGCGCGCACTGGGCCGCC 25080
      W  H  H  D  L  L  T  A  E  D  A  A  P  D  R  A  H  W  A  A

25081 CACACCGCCACCGCCGGCACCGGGCCGCTCCCCGGCGTCGTACGGCCCGGCGCCGCCCCG 25140
      H  T  A  T  A  G  T  G  P  L  P  G  V  V  R  P  G  A  A  P

25141 GGTCCGTGGCGGGCGCGGGAGTGGGAACTGCCCGCCGAACTGGTGGCGGGGATCGACGGC 25200
      G  P  W  R  A  R  E  W  E  L  P  A  E  L  V  A  G  I  D  G

25201 GTCGCCGGGAAGCTGTCCACCGATCCCGCCACCGTGCTGCACGCCGCCTTCCGTATCGCG 25260
      V  A  G  K  L  S  T  D  P  A  T  V  L  H  A  A  F  R  I  A

25261 GTCTGGCGGCTCGCCGGCGAGCGGAACCTGCCCGTCGCCCTCACTCGTGACGGCCGTTCC 25320
      V  W  R  L  A  G  E  R  N  L  P  V  A  L  T  R  D  G  R  S

25321 CACCCCGAACTCCGCACCGCGATCGGCGCCTTCGAGCGTGAGCTCCCGCTCGTCCACGAG 25380
      H  P  E  L  R  T  A  I  G  A  F  E  R  E  L  P  L  V  H  E

25381 ATCCGTCACGAGACGGCGTTCGCGGAATACGCGCGCGCTCTGGACGCGCTCGTCGCCGAG 25440
      I  R  H  E  T  A  F  A  E  Y  A  R  A  L  D  A  L  V  A  E

25441 GGCGAGGAACTCCTCGACCATTGCGACCCGGAACTGCTCGGCAGCCTCGACGGCACCGCG 25500
      G  E  E  L  L  D  H  C  D  P  E  L  L  G  S  L  D  G  T  A

25501 GAAGGGCCCTGCTTCACCTTCACCCACCACCAGGCCGAAACACCGGTCCGGCGGGCCGGC 25560
      E  G  P  C  F  T  F  T  H  H  Q  A  E  T  P  V  R  R  A  G

25561 ATCACCTTTACCACCGTCCATCAGGATTCGGGTACGCCGATTCCCGTCCGCCTGACCGCC 25620
      I  T  F  T  T  V  H  Q  D  S  G  T  P  I  P  V  R  L  T  A

25621 CGACGCGACGGCGCCCGGCTGCGCATGGAACTGGGATACGACGAGGGCCGTATCGACGAG 25680
      R  R  D  G  A  R  L  R  M  E  L  G  Y  D  E  G  R  I  D  E

25681 ACGTTTCCCGAGAACGCCGCCGCCTGCCTCACCCGCATTCTCGAAGGCGTCGTCTCCGCC 25740
      T  F  P  E  N  A  A  A  C  L  T  R  I  L  E  G  V  V  S  A

25741 CCCGAGGGCCCGGTCGGCGACATCCGCATGCTGTCGGACGAGACCGCACGGCTGCTCCGG 25800
      P  E  G  P  V  G  D  I  R  M  L  S  D  E  T  A  R  L  L  R

25801 GAAGCGGGGCTGGGCCCCCGCGTGGAACTTCCCGGCAAGGCGGTCCACGAACTCTTCGCC 25860
      E  A  G  L  G  P  R  V  E  L  P  G  K  A  V  H  E  L  F  A

25861 GAGCAGGCCGCGCGCACCCCCGGGGCGGTCGCGGTCAGCGCGGGCGAGGACGCCCTCACG 25920
      E  Q  A  A  R  T  P  G  A  V  A  V  S  A  G  E  D  A  L  T

25921 TACGCCGAACTCGACGAGCGGTCCAACCGCCTGGCACACCACCTGACCGGGCTCGGGGTG 25980
      Y  A  E  L  D  E  R  S  N  R  L  A  H  H  L  T  G  L  G  V

25981 ACACCCGGCCGGCACGTCGTGGTCTCGGTCGGCCGCTCCGCCGAGCTGCTCGTCGGGCTG 26040
      T  P  G  R  H  V  V  V  S  V  G  R  S  A  E  L  L  V  G  L

26041 CTCGGCGTGCTCAAGGCGGGTGGCGCCTTCGTCCCCGTCGACGTGGGCTTCCCCCGCAAA 26100
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        L  G  V  L  K  A  G  G  A  F  V  P  V  D  V  G  F  P  R  K
26101   CGGCTGGAGTTCGTGCTCCGGGAGACCGCCGCGCCGGTCCTGCTCTGCACCGCCGACGTA   26160
        R  L  E  F  V  L  R  E  T  A  A  P  V  L  L  C  T  A  D  V
26161   CGGGACCGCATCGGCACTCGGACCCTCGACGACGCCGGGGTGACACCCGTCGCGCTGGAC   26220
        R  D  R  I  G  T  R  T  L  D  D  A  G  V  T  P  V  A  L  D
26221   GCCGACCGGCGGCGCATCGCCGCACACCCCGCCGGCCCCACCGGCATCGCCACCACCCCC   26280
        A  D  R  R  R  I  A  A  H  P  A  G  P  T  G  I  A  T  T  P
26281   GACGCCCCCGCGTACGTCGTCTACACCTCCGGCACCACCGGGAAGCCCAACGGCGTACGC   26340
        D  A  P  A  Y  V  V  Y  T  S  G  T  T  G  K  P  N  G  V  R
26341   GTCCCGCACCGGGGCCTCACCAACTACCTCACCTGGTGCACCGGCGCCTACGGACTCGAC   26400
        V  P  H  R  G  L  T  N  Y  L  T  W  C  T  G  A  Y  G  L  D
26401   GGGGGCACCGGCACCCTCGTGCACACCTCCATCAGCTTCGACCTCACCCTCACCACCCTG   26460
        G  G  T  G  T  L  V  H  T  S  I  S  F  D  L  T  L  T  T  L
26461   TTCGGCCCCCTGCTCGCCGGCGGGCAGGTGGTCATGCTCTCCGAGACCGCCGGCGTGACC   26520
        F  G  P  L  L  A  G  G  Q  V  V  M  L  S  E  T  A  G  V  T
26521   GGCCTGATCGCCGCGCTGCGCTCCCGGCGCGACCTCACCCTGGTCAAGCTGACCCCGACC   26580
        G  L  I  A  A  L  R  S  R  R  D  L  T  L  V  K  L  T  P  T
26581   CACCTCGACGTCGTCAACCAGCTGCTCACCCCCGACGAGCTGCGCGGCGCGGTCCGCACC   26640
        H  L  D  V  V  N  Q  L  L  T  P  D  E  L  R  G  A  V  R  T
26641   CTCGTCGTCGGCGGGGAGGCGGTGCGGGCGGAGAGCCTGGAGCCGTTCCGGGCCTCCGGG   26700
        L  V  V  G  G  E  A  V  R  A  E  S  L  E  P  F  R  A  S  G
26701   ACGCGGGTCGTCAACGAGTACGGGCCCAGCGAGACGGTCGTCGGCAGCGTCGCGCACGTC   26760
        T  R  V  V  N  E  Y  G  P  S  E  T  V  V  G  S  V  A  H  V
26761   GTCGACGCCGCCACGCCCCGTACCGGCCCGGTGCCCATCGGCCGGCCGATCGCCAACACC   26820
        V  D  A  A  T  P  R  T  G  P  V  P  I  G  R  P  I  A  N  T
26821   ACCGTCCACCTGCTCGACCAGCGGCGGCGGCCCGTCCCCGACGGCGTCGTCGGCGAGCTG   26880
        T  V  H  L  L  D  Q  R  R  R  P  V  P  D  G  V  V  G  E  L
26881   TGGATCGGCGGCGCCGGTGTCGCCGACGGCTACCTGGGGCGGCCGGAACTCACCGGCGAG   26940
        W  I  G  G  A  G  V  A  D  G  Y  L  G  R  P  E  L  T  G  E
26941   CGCTTCCTCCCCAGCGACTACCCGCCGGACGGCGGCCGGGTCTACCGCACCGGCGACCTG   27000
        R  F  L  P  S  D  Y  P  P  D  G  G  R  V  Y  R  T  G  D  L
27001   GCCCGCCGGCGCGCCGACGGCACCCTGGAGTACCTCGGGCGCACCGACGCGCAGGTGAAG   27060
        A  R  R  R  A  D  G  T  L  E  Y  L  G  R  T  D  A  Q  V  K
27061   ATCCGCGGCGTCCGGGTGGAGCCCGCCGAGACCGAGGCCGTCCTCGCCTCCCACCCCGGC   27120
        I  R  G  V  R  V  E  P  A  E  T  E  A  V  L  A  S  H  P  G
27121   GTCGGCCAGGCCGTCGTGGTCGCCCGGCTGGACGAGGACCCCGGCCGTTCGTCGCCGCTC   27180
        V  G  Q  A  V  V  V  A  R  L  D  E  D  P  G  R  S  S  P  L
27181   GCCGGCGAGCTGACGCTGACCGGCTACGTGGTCCCGGCCCGCGGTGCCCAGGCGCCCCCG   27240
        A  G  E  L  T  L  T  G  Y  V  V  P  A  R  G  A  Q  A  P  P
27241   CACGAGGAGCTCATCGCGTACTGCCGGGAGCGGCTGCCCGAGCACTTCGTCCCGGCCGTC   27300
        H  E  E  L  I  A  Y  C  R  E  R  L  P  E  H  F  V  P  A  V
27301   CTCGTCACCCTCGACGCCCTGCCCGTCACCGGCCACGGCAAGATCGACCGCGGTGCGCTG   27360
        L  V  T  L  D  A  L  P  V  T  G  H  G  K  I  D  R  G  A  L
27361   CCCAAGCCGCACGCCCGGGCCCGGGACGGCGCGGCGTACGTCGCGCCGCGCACCGCCACC   27420
        P  K  P  H  A  R  A  R  D  G  A  A  Y  V  A  P  R  T  A  T
27421   GAGGAGATCCTCGCGGCCACCGTCGCGAAGGTGCTGGGCGTCGAGCGCGTCGGCATCGAC   27480
        E  E  I  L  A  A  T  V  A  K  V  L  G  V  E  R  V  G  I  D
27481   GACAACTACTTCGTCCTGGGCGGCGACTCCATCCGCAGCGTCATGGTCGCCAGCCGGGCC   27540
        D  N  Y  F  V  L  G  G  D  S  I  R  S  V  M  V  A  S  R  A
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27541 CAGGCCCGCGGGGTCGAGGTCACCGTGGCCGACCTGCACCGGCACCCCACCGTCCGGGCC 27600
      Q  A  R  G  V  E  V  T  V  A  D  L  H  R  H  P  T  V  R  A

27601 TGCGCCGCGCACCTGGACGCCCGCGAGGACCTGCCGCGGACGCCCGTCACCGAACCCTTC 27660
      C  A  A  H  L  D  A  R  E  D  L  P  R  T  P  V  T  E  P  F

27661 GCGCTGATCTCCGCCGAGGACCGGGCGCTGGTGCCGGACGACGTCGAGGACGCCTTCCCG 27720
      A  L  I  S  A  E  D  R  A  L  V  P  D  D  V  E  D  A  F  P

27721 CTGAACCTGCTCCAGGAAGGCATGATCTTCCACCGCGACTTCGCGGCGAAGTCGGCCGTC 27780
      L  N  L  L  Q  E  G  M  I  F  H  R  D  F  A  A  K  S  A  V

27781 TACCACGCCATCGCGTCCGTGCGGCTGCGCGCCCCGTTCGACCTCGCCGTGCTGCGGATG 27840
      Y  H  A  I  A  S  V  R  L  R  A  P  F  D  L  A  V  L  R  M

27841 GTCGTGCGCCAGCTCGTCGAGCGGCACCCGATGCTGCGCACCTCCTTCGACATGAGCCGC 27900
      V  V  R  Q  L  V  E  R  H  P  M  L  R  T  S  F  D  M  S  R

27901 TTCAGCCGCCCGCTGCAACTGGTGCACCGCGAGTTCGCCGATCCGCTGCACTACGAGGAC 27960
      F  S  R  P  L  Q  L  V  H  R  E  F  A  D  P  L  H  Y  E  D

27961 CTGCGCGGCAGGAGCGCCGAGGAGCAGGACGCCCGCGTCGAGGAGTGGATCGAGCGGGAG 28020
      L  R  G  R  S  A  E  E  Q  D  A  R  V  E  E  W  I  E  R  E

28021 AAGGAACGCGGCTTCGAGCTGCACGAGTTCCCGCTGATCCGCTTCATGGCGCAGCGCCTG 28080
      K  E  R  G  F  E  L  H  E  F  P  L  I  R  F  M  A  Q  R  L

28081 GAGGACGACGTCTTCCAGTTCACCTACGGCTTCCACCACGAGATCGTGGACGGCTGGAGC 28140
      E  D  D  V  F  Q  F  T  Y  G  F  H  H  E  I  V  D  G  W  S

28141 GAAGCCCTGATGATCACCGAGCTGTTCAGCCACTACTTCTCGGTGATCTACGACGAGCCG 28200
      E  A  L  M  I  T  E  L  F  S  H  Y  F  S  V  I  Y  D  E  P

28201 ATCGCGATCAAGCCACCCACCGCCGGCATGCGCGACGCCGTCGCCCTGGAGCTGGAGGCC 28260
      I  A  I  K  P  P  T  A  G  M  R  D  A  V  A  L  E  L  E  A

28261 CTCGCGGACCGCCGCAACTACGAGTTCTGGGACTCCTACCTCGCCGACGCCACCCTGATG 28320
      L  A  D  R  R  N  Y  E  F  W  D  S  Y  L  A  D  A  T  L  M

28321 CGGCTGCCCAGGCCCGGCACCGGACCCCGGGCCGACAAGGGCGACCGGGACATCACCCGC 28380
      R  L  P  R  P  G  T  G  P  R  A  D  K  G  D  R  D  I  T  R

28381 ATCGCCGTCCCCGTCCCCACCGAACTCTCCGACGGCCTCAAGCGGGTCGCCGCCACCCAC 28440
      I  A  V  P  V  P  T  E  L  S  D  G  L  K  R  V  A  A  T  H

28441 GCCGTCCCGCTGAAGACCGTGCTCCTGGCCGCGCACATGGTGGTGATGTCCCTCTACGGC 28500
      A  V  P  L  K  T  V  L  L  A  A  H  M  V  V  M  S  L  Y  G

28501 GGCCACGAGGACACCCTCACCTACACCGTCACCAACGGCCGCCCCGAGACCGCCGACGGC 28560
      G  H  E  D  T  L  T  Y  T  V  T  N  G  R  P  E  T  A  D  G

28561 AGCACCGCGATCGGGCTGTTCGTCAACAGCCTCGCGCTCCGCGTCCGGATGACCGGCGGC 28620
      S  T  A  I  G  L  F  V  N  S  L  A  L  R  V  R  M  T  G  G

28621 ACCTGGGCCGACCTGATCACCGCCACGCTGGAGTCCGAGCGCGCCTCGATGCCGTACCGG 28680
      T  W  A  D  L  I  T  A  T  L  E  S  E  R  A  S  M  P  Y  R

28681 CGGCTGCCGATGGCCGAACTCAAGCGCCACCAGGGCAACGAACCCCTGGCCGAGACGCTG 28740
      R  L  P  M  A  E  L  K  R  H  Q  G  N  E  P  L  A  E  T  L

28741 TTCTTCTTCACCAACTACCACGTCTTCCACGTGCTCGACCGCTGGATCGACCGCGGCGTC 28800
      F  F  F  T  N  Y  H  V  F  H  V  L  D  R  W  I  D  R  G  V

28801 GGCCACGTCGCCAACGAGCTCTACGGCGAGTCCACCTTCCCCTTCTGCGGCATCTTCCGC 28860
      G  H  V  A  N  E  L  Y  G  E  S  T  F  P  F  C  G  I  F  R

28861 CTGAACCGGGAGACCGGCGAGCTGGAGGTCCGCATCGAGTACGACAGCCTGCAGTTCTCC 28920
      L  N  R  E  T  G  E  L  E  V  R  I  E  Y  D  S  L  Q  F  S

28921 GACGCCCTCATGGAGAGCGTCCGCGACAGCTACGCCCGCGTCCTCGCGGCCCTGGTCGCC 28980
      D  A  L  M  E  S  V  R  D  S  Y  A  R  V  L  A  A  L  V  A

28981 GACCCCGACGGGCGCTACGACCGGCACGAGTTCCGCTCCGACCGCGACCGGGCCGCACTG 29040
      D  P  D  G  R  Y  D  R  H  E  F  R  S  D  R  D  R  A  A  L
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29041 GCCGTCCTCACCCGCGGGCCCGAGGCGCCGGCGGCCGACCGGTGCCTGCACGACCTGGTG 29100
      A  V  L  T  R  G  P  E  A  P  A  A  D  R  C  L  H  D  L  V

29101 GCGGACCGGGCGGCGGACCGCCCCGACGCCCCGGCCGTCCAGCTGGACACCGACGTGCTC 29160
      A  D  R  A  A  D  R  P  D  A  P  A  V  Q  L  D  T  D  V  L

29161 AGCTACGGCGAGCTCGACCGCCGCGCCAACCGGCTGGCCCACCACCTGCGTTCGCTCGGC 29220
      S  Y  G  E  L  D  R  R  A  N  R  L  A  H  H  L  R  S  L  G

29221 ATCGGCCCGGAGAGCGTCGTCGGCGTCCTGGCCGAACGCTCCCTCGCCCAGATCATCGGC 29280
      I  G  P  E  S  V  V  G  V  L  A  E  R  S  L  A  Q  I  I  G

29281 CTCCTCGCGGTCCTCAAGGCGGGCGCCGCCTACGTCCCGCTCGACCCGGCCCAGCCCGAC 29340
      L  L  A  V  L  K  A  G  A  A  Y  V  P  L  D  P  A  Q  P  D

29341 GAGCGCCTCGCCGCCGTCATCGCCGGGAGCGGGGCCGCCGCCGTCCTCCACCGGCCCGGC 29400
      E  R  L  A  A  V  I  A  G  S  G  A  A  A  V  L  H  R  P  G

29401 CTCGAAGGGCGGCTGCCCGCGGGCGTCCGCGCGCTCCCCACCGACGCCGCCGACGGCAGC 29460
      L  E  G  R  L  P  A  G  V  R  A  L  P  T  D  A  A  D  G  S

29461 ACCGCCACGCACGACCCCGGGCCCACCGCCACGCCCCGCAACGCCGCGTACGTGATGTAC 29520
      T  A  T  H  D  P  G  P  T  A  T  P  R  N  A  A  Y  V  M  Y

29521 ACCTCCGGATCCACCGGAGAGCCCAAGGGCATCGTCGTCGAACACCGCAACGTCGTGGCC 29580
      T  S  G  S  T  G  E  P  K  G  I  V  V  E  H  R  N  V  V  A

29581 TCCCTCGCCGCCCGCGGCGCCCACTACGCGGCCGGACCCGGCCGGTTCCTGCTGCTGTCC 29640
      S  L  A  A  R  G  A  H  Y  A  A  G  P  G  R  F  L  L  L  S

29641 TCCTTCGCCTTCGACAGCTCGGTCGCCGGCATCTTCTGGACGCTGACCCAGGGCGGCACC 29700
      S  F  A  F  D  S  S  V  A  G  I  F  W  T  L  T  Q  G  G  T

29701 CTCGTCCTGCCCGGCGAGGGACAGCAACTCGACCCCGCCGCGCTGGTGGAGACCATCGCC 29760
      L  V  L  P  G  E  G  Q  Q  L  D  P  A  A  L  V  E  T  I  A

29761 CGGCAACGGCCCACCCACACCCTCGCCATCCCCTCCCTGCTGGCGCCCGTCCTGGACCAG 29820
      R  Q  R  P  T  H  T  L  A  I  P  S  L  L  A  P  V  L  D  Q

29821 GCCGCCCCCGGCGACCTCGCCTCCCTGCGCACGGTGATCGCCGCGGGCGAGTCCTGTCCG 29880
      A  A  P  G  D  L  A  S  L  R  T  V  I  A  A  G  E  S  C  P

29881 GCCGAACTGGCCGCCGCCTGCCGGGACCTGCTGCCCGGGAGCACCTTCCACAACGAGTAC 29940
      A  E  L  A  A  A  C  R  D  L  L  P  G  S  T  F  H  N  E  Y

29941 GGCCCCACCGAGACCACCGTGTGGAGCACCGTCTGGTCCCAGGAGAACGAGCACGACGGA 30000
      G  P  T  E  T  T  V  W  S  T  V  W  S  Q  E  N  E  H  D  G

30001 CCCCACCTCCCCATCGGCCGGCCGGTCGCGGGCACCTGGGTGCACCCCCGCGACCACCGC 30060
      P  H  L  P  I  G  R  P  V  A  G  T  W  V  H  P  R  D  H  R

30061 GGACGCACCGTCCCCCTCGGCGTCGCCGGCGAACTCTCCATCGGCGGCGCCGGCGTGGCC 30120
      G  R  T  V  P  L  G  V  A  G  E  L  S  I  G  G  A  G  V  A

30121 CGCGGCTACCTCGGGCGCCCCCGGGACACCGCGGCCGCCTTCCGCCCCGACCCCGAGGCC 30180
      R  G  Y  L  G  R  P  R  D  T  A  A  A  F  R  P  D  P  E  A

30181 ACGGCTCCCGGCGGCCGCGCCTACGCCACCGGCGACCTCGGCCGCTACCTCCCCGACGGC 30240
      T  A  P  G  G  R  A  Y  A  T  G  D  L  G  R  Y  L  P  D  G

30241 AACCTGGAGTTCCTCGGCCGCGCCGACCACCAGGTCAAGATCCGCGGCTTCCGGGTCGAG 30300
      N  L  E  F  L  G  R  A  D  H  Q  V  K  I  R  G  F  R  V  E

30301 CTCGGCGAGATCGAGGCCGTCCTCGACACCCACCCGGAGCTCCAGCGGACCATCGTCATG 30360
      L  G  E  I  E  A  V  L  D  T  H  P  E  L  Q  R  T  I  V  M

30361 GCACGCGGCGACCACCCCGGCGACCAGGTGCTCGTCGCCTACGTCCTCCCCGCCCCCGGC 30420
      A  R  G  D  H  P  G  D  Q  V  L  V  A  Y  V  L  P  A  P  G

30421 CGGCGGCCCGAACCCGCCGACATCCAGGGGTACGTCCGCGACCGGCTGCCCCGCTACATG 30480
      R  R  P  E  P  A  D  I  Q  G  Y  V  R  D  R  L  P  R  Y  M

30481 GTGCCCACCGCGGTGATCGTCCTCGACGCGGTACCGCTGACCGCCGCCGGCAAGGTCGAC 30540
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          V  P  T  A  V  I  V  L  D  A  V  P  L  T  A  A  G  K  V  D
30541 CGGGCCTCGCTCCCCGCCCCCAGCCACGCCCAGCTCACCCGGGACCAGGAGTACGTCGAG 30600
          R  A  S  L  P  A  P  S  H  A  Q  L  T  R  D  Q  E  Y  V  E
30601 CCCGGCACCGACACCGAGCGGGCGCTCGCCGCCATCTGGGCCGACGTCCTCAAACTGGAC 30660
          P  G  T  D  T  E  R  A  L  A  A  I  W  A  D  V  L  K  L  D
30661 CGGATCGGGGCCGGTGACCGCTTCTTCGACGTCGGCGGCGAATCCCTGCGCGCGATGCAG 30720
          R  I  G  A  G  D  R  F  F  D  V  G  G  E  S  L  R  A  M  Q
30721 GCCACCGCCGCGGCCAACAAGATGTTCCGCACCCGCGTCTCCGTCCGCCGCCTCTTCGAG 30780
          A  T  A  A  A  N  K  M  F  R  T  R  V  S  V  R  R  L  F  E
30781 GCGCCCTCCCTGCGGGAGTTCGCCCACGAGATCGACAAGGCCCGCCTCGCGGGCGGCGGG 30840
          A  P  S  L  R  E  F  A  H  E  I  D  K  A  R  L  A  G  G  G
30841 ACCGGCCTCACCGGCCCCGCGGCCGCCCCGGCCACCGGAGGTGCCGCCGAATGACCCCGG 30900
          T  G  L  T  G  P  A  A  A  P  A  T  G  G  A  A  E  *
                                                      M  T  P  A  (orf25)

30901 CCGCCGACACCACCCACCCGCTCTCGCCGGCCCAGCGCAGCATGTGGTTCCTGCACCGGC 30960
          A  D  T  T  H  P  L  S  P  A  Q  R  S  M  W  F  L  H  R  L
30961 TCGCGCCCGAGGTGCCCGCCTACAACATCTGCACCGCCATCGAGCTCACCGGCACACCGC 31020
          A  P  E  V  P  A  Y  N  I  C  T  A  I  E  L  T  G  T  P  R
31021 GCCCGGCGGCGCTGCGGGACGTGGTACGGCGGCTCGGCCGCAGGCACGAGGCGCTGCGCA 31080
          P  A  A  L  R  D  V  V  R  R  L  G  R  R  H  E  A  L  R  T
31081 CGGTGTTCCCGTCGGTGGGGGAGACCCCCCGCCAACGGGTCACCGACCGGGCGGCGCCCC 31140
          V  F  P  S  V  G  E  T  P  R  Q  R  V  T  D  R  A  A  P  L
31141 TGCGGACCGTGGACCTCACCCACCTGACCCCCGCCGCCGCCGAGGCCGAGACCGCACGGA 31200
          R  T  V  D  L  T  H  L  T  P  A  A  A  E  A  E  T  A  R  T
31201 CGCTACGGTGCGCCGCCGCCCGGCCGTTCCGGCTCGACACCGGCCCCCTGGCGGAATGGA 31260
          L  R  C  A  A  A  R  P  F  R  L  D  T  G  P  L  A  E  W  T
31261 CCCTGCTGCGCCGCGCCCCCGGCCACGCGCTGCTCGTCCTCTCCGTCCACCACATCGTCT 31320
          L  L  R  R  A  P  G  H  A  L  L  V  L  S  V  H  H  I  V  F
31321 TCGACGGCGGCTCGCTCCACGTGGTCTGCCGCGAACTGGAGGAGGCGTACGGAGCGGCCC 31380
          D  G  G  S  L  H  V  V  C  R  E  L  E  E  A  Y  G  A  A  L
31381 TCGCCGGGCGCCCGGACCCCCTCGGCACACCCGCGCCGGGCTACGGACGGCAGTGCCGGA 31440
          A  G  R  P  D  P  L  G  T  P  A  P  G  Y  G  R  Q  C  R  T
31441 CGCGGGCGGCGGAACAGGACGAGGCCGGGCGGGAGTTCTGGCGCCGCGAACTGTCCGGCG 31500
          R  A  A  E  Q  D  E  A  G  R  E  F  W  R  R  E  L  S  G  A
31501 CGCCACCCCGCACGACCGTCTTCCGGGGCACCGGCCGGCCCGCCGGACCGCCCGCCCGCG 31560
          P  P  R  T  T  V  F  R  G  T  G  R  P  A  G  P  P  A  R  A
31561 CCACCGTCCACTACGGCACCGACGATCCGGCCCCGACCGCGGACTTCTGCCGCGAGCACG 31620
          T  V  H  Y  G  T  D  D  P  A  P  T  A  D  F  C  R  E  H  A
31621 CCGTCACCGGCTACGTGCTGCTGCTCGCGGCCCTCGCCTGCCTGGTCGCCCGGTACACCG 31680
          V  T  G  Y  V  L  L  L  A  A  L  A  C  L  V  A  R  Y  T  G
31681 GCCGGACGGACGTGGTGATCGGCTCACCCGTCGGACTGCGCGAGGACCCCGAAGGGCTCG 31740
          R  T  D  V  V  I  G  S  P  V  G  L  R  E  D  P  E  G  L  A
31741 CCACCGTCGGCCCGATGCTCAACCTGCTGCCGCTGCGCCTCCGGCTGCACGGCGACCCCG 31800
          T  V  G  P  M  L  N  L  L  P  L  R  L  R  L  H  G  D  P  G
31801 GCTTCGGCGAGGTCCTGGCCCGCACCCGGGAGACGCTGCTCGGCGCGCTGGAGCACCGCA 31860
          F  G  E  V  L  A  R  T  R  E  T  L  L  G  A  L  E  H  R  T
31861 CCACACCGTTCGAGGACATCGTCGACGCGGTGGGCGCCGACCGGGACCCGGACGTCAGCC 31920
          T  P  F  E  D  I  V  D  A  V  G  A  D  R  D  P  D  V  S  P
31921 CCCTCTTCCAGATCCTCTTCGCCCACGAACGCCCCCCGGCCCCACCCGCGTTACCGGGCG 31980
          L  F  Q  I  L  F  A  H  E  R  P  P  A  P  P  A  L  P  G  V
                                                                  q
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31981 TCCGTGCCCGCGTCGTACCCGTCCCCGCTCCGGCCGCCAAGTACGAGCTCGCCGTCACCG 32040
        R  A  R  V  V  P  V  P  A  P  A  A  K  Y  E  L  A  V  T  A

32041 CCACCGAGACGCCCGACGGGCTCCGGCTGATCGTCGAGGCGGAGCACGGACACGGGGAAC 32100
        T  E  T  P  D  G  L  R  L  I  V  E  A  E  H  G  H  G  E  P

32101 CGGCCGAACTCGCCGCCTTCGCCCGCCACTTCGGCGTCCTGCTGGCCGCCGGGGTCCGCG 32160
        A  E  L  A  A  F  A  R  H  F  G  V  L  L  A  A  G  V  R  A

32161 CGCCGGACACACCGCTGAGCCGCCTGCCGCTGCTCACCGACGAGGAGCGGCGCCGGCTCA 32220
        P  D  T  P  L  S  R  L  P  L  L  T  D  E  E  R  R  R  L  T

32221 CCGACACCACGGCCCCCCGCACCGCGCCGGAGGCCCCCTACCGCCCCCTGCACCGGCTGG 32280
        D  T  T  A  P  R  T  A  P  E  A  P  Y  R  P  L  H  R  L  V

32281 TCGAGGAGTCCGCCGCCCGCCGGCCCGACGCCCTGGCGGTCGTCGGCGGCACGCGTCACC 32340
        E  E  S  A  A  R  R  P  D  A  L  A  V  V  G  G  T  R  H  L

32341 TCAGCTACCGGGAGCTGAACTGCCGCGCCAACCGGCGTGCCGCCTGGCTGCGCCGCGCTG 32400
        S  Y  R  E  L  N  C  R  A  N  R  R  A  A  W  L  R  R  A  G

32401 GCATCGGCACCGAGGACGTGGTCGGCGTCCGGCTGGAACGCGGCCCGGAACTCCTCGTCT 32460
        I  G  T  E  D  V  V  G  V  R  L  E  R  G  P  E  L  L  V  S

32461 CGCTCCTCGCCGTCCTCAAGGCCGGCGCCGCCTACCTGCCCGTCGACCCGGCGCTGCCCG 32520
        L  L  A  V  L  K  A  G  A  A  Y  L  P  V  D  P  A  L  P  A

32521 CCGAGCGGGTACGGCTGATGCTCGACGACGCCCGGGCCGCGCTGCTGCTCACCGAGACCG 32580
        E  R  V  R  L  M  L  D  D  A  R  A  A  L  L  L  T  E  T  A

32581 CGCTCGGCACCCCGCCGGCCCCGGCCGGCACCCCCGTGCACCACGTGGACGGACCGCCAC 32640
        L  G  T  P  P  A  P  A  G  T  P  V  H  H  V  D  G  P  P  P

32641 CGCCGACCCGGCCCGGGGACGACGCCGACCACACCGGCCCCGACCTGCCCACCAGCCTCG 32700
        P  T  R  P  G  D  D  A  D  H  T  G  P  D  L  P  T  S  L  A

32701 CCTACCTCCTCTACACCTCCGGGTCGACGGGCCGGCCCAAGGCCGTGGCCCTCCAGCACG 32760
        Y  L  L  Y  T  S  G  S  T  G  R  P  K  A  V  A  L  Q  H  D

32761 ACAGCGCCGCGGCGTTCCTGCGCTGGGCGGGCCGCGCCTTCGACGGCGGGGAGCTGGCCG 32820
        S  A  A  A  F  L  R  W  A  G  R  A  F  D  G  G  E  L  A  A

32821 CCGTCCTGGCCACCACCTCCGCCGGCTTCGACCTGTCGGTCTTCGAGCTGTTCGCCCCCC 32880
        V  L  A  T  T  S  A  G  F  D  L  S  V  F  E  L  F  A  P  L

32881 TGGCCCACGGCGGCACCGTCGTCCTCGCCGACAGCGCCCTGCACGTGCCCGCCCTGCCCT 32940
        A  H  G  G  T  V  V  L  A  D  S  A  L  H  V  P  A  L  P  W

32941 GGGCGCCCGCGGCGACGCTCCTGAACACCGTGCCCTCCGCGGCCGCCGCCCTGCTGGACG 33000
        A  P  A  A  T  L  L  N  T  V  P  S  A  A  A  A  L  L  D  A

33001 CCGACGGCCTGCCCGACGGTCTGACGGCCGTCAACCTGGCGGGCGAGCCCCTGACCGCGG 33060
        D  G  L  P  D  G  L  T  A  V  N  L  A  G  E  P  L  T  A  E

33061 AGCTGGTCGCCCGGCTGCACGCCCGCCTGCCGAAGGCCGCCGTCCGCAACCTCTACGGCC 33120
        L  V  A  R  L  H  A  R  L  P  K  A  A  V  R  N  L  Y  G  P

33121 CCTCGGAGGCCACCACCTACGCCACCGCGGCCCTCGTGCCCGCGGGCGGCACCGAGGCGC 33180
        S  E  A  T  T  Y  A  T  A  A  L  V  P  A  G  G  T  E  A  P

33181 CGGCCATCGGCCGGGCGCTCGGCGCGGCCCGCGTGTGGACCGCCGACGACCGGCAGCGCC 33240
        A  I  G  R  A  L  G  A  A  R  V  W  T  A  D  D  R  Q  R  P

33241 CCCTCCCCGGCGCGGTCGTCGGTGAACTCCTCATCGGCGGTACGGCCCCGGCCCGCGGCT 33300
        L  P  G  A  V  V  G  E  L  L  I  G  G  T  A  P  A  R  G  Y

33301 ACCTCGGCCGGCCGGGACCGACCGCCGACGCCTTCCGGCCCGATCCGACGGGACCGCCCG 33360
        L  G  R  P  G  P  T  A  D  A  F  R  P  D  P  T  G  P  P  G

33361 GCTCCCGGCTCTACCGCACCGGGGACCTGGCCGTACGCCGCCCCGACGGCCGGTTCGTGT 33420
        S  R  L  Y  R  T  G  D  L  A  V  R  R  P  D  G  R  F  V  F

33421 TCCTCGGCCGCAAGGACGAGCAGATCAAACTCCGCGGGGTGCGCATCGAACCGGGCGAGG 33480
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          L  G  R  K  D  E  Q  I  K  L  R  G  V  R  I  E  P  G  E  V
33481 TGGAAGCCGCTCTCCGCCAGTGCGCGCCGGTCGCCGCGGCCGCCGTCGTGCTCGCCGGGA 33540
          E  A  A  L  R  Q  C  A  P  V  A  A  A  A  V  V  L  A  G  T
33541 CCACCGCGGAGAACCACCGCCTCGTCGGCTTCGTCACCCCTTCGCCCGGCGCCCGCGTCG 33600
          T  A  E  N  H  R  L  V  G  F  V  T  P  S  P  G  A  R  V  D
33601 ACCCCGAGCGCACCCTCGCCGCGCTGCGTTCGCGCCTGCCCGCCGCCCTCGTGCCCGCCG 33660
          P  E  R  T  L  A  A  L  R  S  R  L  P  A  A  L  V  P  A  A
33661 CGCTGGTGGTGTGCGACGCCCTGCCGCTGACCGCCAACGGGAAGACCGACCGGGCCGCCC 33720
          L  V  V  C  D  A  L  P  L  T  A  N  G  K  T  D  R  A  A  L
33721 TCGCCCGGCGGGCGCGCGGACACCGGCCGGACCACGGCGCGTACGCCCCGCCCCGCACCC 33780
          A  R  R  A  R  G  H  R  P  D  H  G  A  Y  A  P  P  R  T  R
33781 GCGTCGAGAAGGCGGTCGCCGCGATCTGGCGCGAGGTGCTCGGGACCGAACGGGTGGGGA 33840
          V  E  K  A  V  A  A  I  W  R  E  V  L  G  T  E  R  V  G  I
33841 TCCACCAGGGGTTCTTCGACGCGGGCGGCACCTCCCTGTCGCTGCTGCGCCTTCACCACC 33900
          H  Q  G  F  F  D  A  G  G  T  S  L  S  L  L  R  L  H  H  R
33901 GGCTGGTCGCGTCCGTCCATCCCGGCCTCCGGCTCGCCGACGTCTTCCGGCTGCCGACCG 33960
          L  V  A  S  V  H  P  G  L  R  L  A  D  V  F  R  L  P  T  V
33961 TCGCCGCGCTCGCCGCGTTCGTGGACGGGCAGGAGGACGCGCGCGAGACGGCCGTCGGCG 34020
          A  A  L  A  A  F  V  D  G  Q  E  D  A  R  E  T  A  V  G  D
34021 ACGCGGCCCTCCGGGCCGGCCGGCGCCGCGCCGCGGTGGCCGCGCGCCGCAGGAAAGGCG 34080
          A  A  L  R  A  G  R  R  R  A  A  V  A  A  R  R  R  K  G  G
34081 GCGGACGATGAGCCATGCCGACGCGGGCGACGGGCTCGACGCGGCTGACACGACTGACGC 34140
                M  S  H  A  D  A  G  D  G  L  D  A  A  D  T  T  D  A   (orf24)
          G  R  *
34141 GGCCGACGGGATCGCCGTGATCTCGCTGGGCGGACGCTTCCCCGGAGCGGACCGGGTGGA 34200
          A  D  G  I  A  V  I  S  L  G  G  R  F  P  G  A  D  R  V  D
34201 CCGCCTCTGGACGAACCTGCTCGACCGCGAGGACGCCATCAGCCACTTCACCGCCGACGA 34260
          R  L  W  T  N  L  L  D  R  E  D  A  I  S  H  F  T  A  D  E
34261 ACGCCTCGCCCGGGGCCGCGACCCCGAACTGGTGCGCCACCCGCGGTTCGTCGGCGCGGA 34320
          R  L  A  R  G  R  D  P  E  L  V  R  H  P  R  F  V  G  A  E
34321 AGGCGTCCTCGGCGACGTCTCCCTCTTCGACGCCGAGTTCTTCGGCTGCTCGCCGCGCGA 34380
          G  V  L  G  D  V  S  L  F  D  A  E  F  F  G  C  S  P  R  E
34381 GGCCGAAGTCATGGACCCGCAGCACCGGCTCTGCCTGGAGGAGGCGTGGCACGTCTTCGA 34440
          A  E  V  M  D  P  Q  H  R  L  C  L  E  E  A  W  H  V  F  D
34441 CACCGCCGGCTACGACCCGGCGGCGACGGGCACCGCGGTCGGGGTGTTCCTCTCCGCGAG 34500
          T  A  G  Y  D  P  A  A  T  G  T  A  V  G  V  F  L  S  A  S
34501 CCTCAGCTCGTACCTGATCCGCAACGTCCTGCCCGGCGGCGCGGCACAGCGCCTGCTCGG 34560
          L  S  S  Y  L  I  R  N  V  L  P  G  G  A  A  Q  R  L  L  G
34561 CGGCTTCCCGCTGCTGATCCACAACGACAAGGACTTTCTGGCCACCACCGTGTCCCACAA 34620
          G  F  P  L  L  I  H  N  D  K  D  F  L  A  T  T  V  S  H  K
34621 ACTGGGCCTCACCGGGCCGAGTTACGCCGTCGGCTCGGCCTGCTCGTCCTCCCTCGTCGC 34680
          L  G  L  T  G  P  S  Y  A  V  G  S  A  C  S  S  S  L  V  A
34681 GGTGCACCTGGCCTGCCAGAGCCTGCTCACCGAGGAATGCGACATGGCGCTGGCCGGCGG 34740
          V  H  L  A  C  Q  S  L  L  T  E  E  C  D  M  A  L  A  G  G
34741 GGTCTCGCTCCAAGTGCCGCAGGGCCAGGGGTACGTGCACGCCGACGACGGCATCTACTC 34800
          V  S  L  Q  V  P  Q  G  Q  G  Y  V  H  A  D  D  G  I  Y  S
34801 ACCCGACGGGCGCTGCGCCCCCTTCGACGCCGGCGCGGCGGGCACGGTGGGCGGCAGCGG 34860
          P  D  G  R  C  A  P  F  D  A  G  A  A  G  T  V  G  G  S  G
34861 CGTGGGCCTCGTCCTGCTCAAGCGGCTCGCCGACGCCGTGCGCGACGGGGACCGCGTCCA 34920
          V  G  L  V  L  L  K  R  L  A  D  A  V  R  D  G  D  R  V  H
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34921 CGCGGTGATCCTCGGCTCGGCGGTGAACAACGACGGCGCCGACAAGGTCGGTTACACGGC 34980
      A  V  I  L  G  S  A  V  N  N  D  G  A  D  K  V  G  Y  T  A

34981 GCCCGGCGTCACCGGCCAGAGCGCCGTCGTCGCCGAGGCCCTGGCGGTGGCCGGGATCTC 35040
      P  G  V  T  G  Q  S  A  V  V  A  E  A  L  A  V  A  G  I  S

35041 CGCCGCGACCGTCGGCGTCCTGGAGGCGCACGGCACCGGCACCCGGCTGGGCGATCCCGT 35100
      A  A  T  V  G  V  L  E  A  H  G  T  G  T  R  L  G  D  P  V

35101 CGAAGTGGCCGCGCTCACCCGGGCGTTCCGCGCCCACACGGACCGCAGCGGCTTCTGCGC 35160
      E  V  A  A  L  T  R  A  F  R  A  H  T  D  R  S  G  F  C  A

35161 GCTGGGCTCGGTGAAGGCCAACGTGGGCCACCTGGACGCGGCGGCGGGCGTCACCGGGCT 35220
      L  G  S  V  K  A  N  V  G  H  L  D  A  A  A  G  V  T  G  L

35221 GATCAAGGCCGTGCTGGCGGTCCGCGAGGGCGTCATCCCCGGCACCCCGCACTACCGTTC 35280
      I  K  A  V  L  A  V  R  E  G  V  I  P  G  T  P  H  Y  R  S

35281 GCCCAACCCCGCCATCGACTTCGCCACCACACCCTTCTACGTCACCGCCGACACCCTCGC 35340
      P  N  P  A  I  D  F  A  T  T  P  F  Y  V  T  A  D  T  L  A

35341 CTGGCCGGAGGCGGACCACCCCCGCCGGGCCGGCGTCAGCTCCTTCGGCATCGGGGGCAC 35400
      W  P  E  A  D  H  P  R  R  A  G  V  S  S  F  G  I  G  G  T

35401 CAACGCCCACGTGATCCTGGAACAGGCCCCGCCGGCCGCCCCCCGCGCGGACCGGACCGC 35460
      N  A  H  V  I  L  E  Q  A  P  P  A  A  P  R  A  D  R  T  A

35461 CGGGGTGCCCATGCCGTTGGTGGTGTCCGCCCGCACCCGCGAAGCACTGGCGGAGGCCGT 35520
      G  V  P  M  P  L  V  V  S  A  R  T  R  E  A  L  A  E  A  V

35521 CCGGGACCTGGCGGCGTGGTCGGCCCCGGAGCCGGGGACCCGGCTCGCCGATCTCGCCGC 35580
      R  D  L  A  A  W  S  A  P  E  P  G  T  R  L  A  D  L  A  A

35581 CACGCTGGCCGGGCGCCGGGCCTTCCCGTACCGCGCCGCCGTCGTGTGCCACGACCTGCC 35640
      T  L  A  G  R  R  A  F  P  Y  R  A  A  V  V  C  H  D  L  P

35641 CGAGGCCGCGCGCCTGCTGGGCGGCGCGCGCGGCGAGACCGCGCTCCCCGGCAGGGAGGC 35700
      E  A  A  R  L  L  G  G  A  R  G  E  T  A  L  P  G  R  E  A

35701 CGTGTTCCTCTTCCCCGGGCAGGGCACCCTCCCGCCGGACACCGGGCGCGGCCTGTACGC 35760
      V  F  L  F  P  G  Q  G  T  L  P  P  D  T  G  R  G  L  Y  A

35761 GGACGTGCCGGCGTTCCGCGCCCACTTCGACGCCTGTGCCGAAGGGTTCGCCCCGCTCGG 35820
      D  V  P  A  F  R  A  H  F  D  A  C  A  E  G  F  A  P  L  G

35821 CACCGACCTCCACGCCGCGCTCGGGGCCCCGGCCGACGACACCAGGGCCGCGCAACCCGC 35880
      T  D  L  H  A  A  L  G  A  P  A  D  D  T  R  A  A  Q  P  A

35881 CCTCTTCGCCGTCGAGTACGCCCTCGCCCGCACCCTGATGGACTGGGGTGTGCGCCCGGC 35940
      L  F  A  V  E  Y  A  L  A  R  T  L  M  D  W  G  V  R  P  A

35941 CGCGATGCTCGGGCACAGCCTCGGCGAGTACGTCGCGGCGACGCTGGCCGGGGTGCTGTC 36000
      A  M  L  G  H  S  L  G  E  Y  V  A  A  T  L  A  G  V  L  S

36001 CCTGCCGGACGCGCTGACGCTCGTCCGGGCCCGGGCGGAAGCGCAGCACACCATGCCGCC 36060
      L  P  D  A  L  T  L  V  R  A  R  A  E  A  Q  H  T  M  P  P

36061 CGGCCGCATGCTCGCGGTCCCGCTCACGCCGGACGACCTGCGCCCGCTGCTGCCCCCGGA 36120
      G  R  M  L  A  V  P  L  T  P  D  D  L  R  P  L  L  P  P  E

36121 GGTGGAGTTCAGCGCCTTCAACGCCCCCGGCCGCTGCGTCGTCGGCGGGCCCCCGGAGCC 36180
      V  E  F  S  A  F  N  A  P  G  R  C  V  V  G  G  P  P  E  P

36181 GGTGGCGGAGCTGCGCGCCCGGCTGGCGCGGCGCGGAGTGCCGGCCGCCGAACTGGCCAC 36240
      V  A  E  L  R  A  R  L  A  R  R  G  V  P  A  A  E  L  A  T

36241 CGCGCACGCCTTCCACTCGGCGGCCGTCGAACCGCTGCTGGACGGCTTCCGGGGCGTGCT 36300
      A  H  A  F  H  S  A  A  V  E  P  L  L  D  G  F  R  G  V  L

36301 GGAAGGCGTCCGACTGCGGCCGCCCCGGCTGCGGTACGTGTCCTCCCTCACCGGCGACTG 36360
      E  G  V  R  L  R  P  P  R  L  R  Y  V  S  S  L  T  G  D  W

36361 GGCCGACGCCGCGGTCACCACCCCCGCGTACTGGCTCGCCCACCTGCGCCGGCCCGTCCG 36420
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          A  D  A  A  V  T  T  P  A  Y  W  L  A  H  L  R  R  P  V  R
36421 CTTCGCCGACGGCCTGCGGCGCTGCCTGGACCTCGGCCCCGTCGCCCTGGTCGAGACCGG 36480
          F  A  D  G  L  R  R  C  L  D  L  G  P  V  A  L  V  E  T  G
36481 GCCGCGGGCCGGACTGACCGGCCTGGCCCGCCGCGCCGCGGGCCCCGGCGAGCCCCCTTA 36540
          P  R  A  G  L  T  G  L  A  R  R  A  A  G  P  G  E  P  P  Y
36541 CACCGTCCGCTGCCTGGCCGCCCCCGACGAGGCGGCTTCGCTGACCCACGCGGTCGCCGT 36600
          T  V  R  C  L  A  A  P  D  E  A  A  S  L  T  H  A  V  A  V
36601 ACTCTGGCGCTCGGGCTGCGCCGTCGACTGGACGGCGTTCCACCGCCCCGGGCGCCCCCG 36660
          L  W  R  S  G  C  A  V  D  W  T  A  F  H  R  P  G  R  P  R
36661 CCGCACCACCGTGCCCGGCTACCCCTTCCAACGGGTACGGCACTGGATCGACGCGCCGGA 36720
          R  T  T  V  P  G  Y  P  F  Q  R  V  R  H  W  I  D  A  P  D
36721 CGAGTCCGAACCCACGGACCTCGCCACCGCCCTGCGCGCGGAGTTGCGGACGGACGGCGA 36780
          E  S  E  P  T  D  L  A  T  A  L  R  A  E  L  R  T  D  G  D
36781 TCCGCCGCTCGCCGTCGATCAGCGGCCCGGACTGCGCACGGGGCTGAACCGGCTGTGCGC 36840
          P  P  L  A  V  D  Q  R  P  G  L  R  T  G  L  N  R  L  C  A
36841 CGCCCTGGCCCGCGACTACCTGGCCACCGGCGTCGAAGCGAGCGGGGTCCTGCCCGGATT 36900
          A  L  A  R  D  Y  L  A  T  G  V  E  A  S  G  V  L  P  G  F
36901 CCACCGCTTCCTGGACTACCTGCGCACCCTGGCCGCCTCCGCACCGGCCGCGGACGACGC 36960
          H  R  F  L  D  Y  L  R  T  L  A  A  S  A  P  A  A  D  D  A
36961 GGGGACGATCGCCGCGGAGATCACCGCGGCCCACCCGTCCTTCTCCGGGCTCGTCGACCT 37020
          G  T  I  A  A  E  I  T  A  A  H  P  S  F  S  G  L  V  D  L
37021 GCTCCGGCACTGCGCCCAGGGCTATCCGCGCGCCCTGTCCACCCCCGGAGCCGCACTGGA 37080
          L  R  H  C  A  Q  G  Y  P  R  A  L  S  T  P  G  A  A  L  D
37081 CGTCCTCTATCCGGCCGGCAGCGGCGACCTCCTGCGCCGCACCCTGGGCGAGGGCACCGC 37140
          V  L  Y  P  A  G  S  G  D  L  L  R  R  T  L  G  E  G  T  A
37141 CGACCACCGCGCCACCGGCCGCCTCACCCGGCTGGCCGGCTCCCTGCTCGACCGGCTCGC 37200
          D  H  R  A  T  G  R  L  T  R  L  A  G  S  L  L  D  R  L  A
37201 GGCCGACCGCGAACCCGGCCGCCCGCTGCGCGTCCTGGAGGCCGGAGCGGGCGCGGGCAG 37260
          A  D  R  E  P  G  R  P  L  R  V  L  E  A  G  A  G  A  G  S
37261 CCTCACCCAGGCCCTGGTCACCCGGGCCCCCGGCCGGCTCGACTACCACGCCACCGACAT 37320
          L  T  Q  A  L  V  T  R  A  P  G  R  L  D  Y  H  A  T  D  I
37321 CTCCCGGCACTTCGTGACCGCACTCGGCCGGGAGGCCGCCCGGCGCGGCCTGGACTTCGT 37380
          S  R  H  F  V  T  A  L  G  R  E  A  A  R  R  G  L  D  F  V
37381 CCGCGCACGCGTCCTCGACATCGCCCGCGACCCAGGCGAACAGGGCTTCGCCGGCGAGCG 37440
          R  A  R  V  L  D  I  A  R  D  P  G  E  Q  G  F  A  G  E  R
37441 GTTCGACGTCGTCTGCGGCCTCGACGTGGTCCACGCCACCCCCGACCTGCGCACCACGCT 37500
          F  D  V  V  C  G  L  D  V  V  H  A  T  P  D  L  R  T  T  L
37501 CGGCCATCTGCGCTCCCTGATGGCACCGGACGGCACCCTCGCGCTGATCGAGACCACCGC 37560
          G  H  L  R  S  L  M  A  P  D  G  T  L  A  L  I  E  T  T  A
37561 CGACGACCCCTGGCTGACGATGATCTGGGGCCTGACGGACGGCTGGTGGCACCACACCGA 37620
          D  D  P  W  L  T  M  I  W  G  L  T  D  G  W  W  H  H  T  D
37621 CCGGCGCACCCACGGCCCGCTGCTCGACGCCGCCGGCTGGCGCGCCCTCCTGGCCGGCGA 37680
          R  R  T  H  G  P  L  L  D  A  A  G  W  R  A  L  L  A  G  E
37681 GGACTTCGCCACGGCCGATGTGATCGTGCCGCCCGACGGCCCCCAGGACGCGGCCCTGCT 37740
          D  F  A  T  A  D  V  I  V  P  P  D  G  P  Q  D  A  A  L  L
37741 GCTCGCCCGGCAGACCCCCCGGCCGGCGGCGGCCGCACCGTCCGTCGGCAAGCGGGACGT 37800
          L  A  R  Q  T  P  R  P  A  A  A  A  P  S  V  G  K  R  D  V
37801 CGGCACGTGGTGCTACGCCCGCGGCTGGCGGCACGCCGCGCCCGCCGACCCCGCCCCGCT 37860
          G  T  W  C  Y  A  R  G  W  R  H  A  A  P  A  D  P  A  P  L
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37861 GACGGGCGGCTGCCTGCTGCTGGGCGACGGGGACACGGCGAAGGCCGTCGCGAGCCGGCT 37920
        T  G  G  C  L  L  L  G  D  G  D  T  A  K  A  V  A  S  R  L

37921 GGAGGCCCTCGGCGTGCCCGTCACCACCGTCGGCGGCGGCCGACCGCCGGGCCCCGAGCG 37980
        E  A  L  G  V  P  V  T  T  V  G  G  G  R  P  P  G  P  E  R

37981 GTACCGGGAACTCGTCGGCCCCGCCACCCGCCTGGCCGTCGACCTGTGGCCGCTGCGCGA 38040
        Y  R  E  L  V  G  P  A  T  R  L  A  V  D  L  W  P  L  R  D

38041 CGCGTCCCACCGCGGCCGCGCCGCCGGCGCCGCCGGCGTACGGACCGCCCAGGACGCCGC 38100
        A  S  H  R  G  R  A  A  G  A  A  G  V  R  T  A  Q  D  A  A

38101 GCTGCACAACCTGCTCCACCTCGCCCGGGCCTTCGGCGCGCTGGAGGAGCGCCACCCCGC 38160
        L  H  N  L  L  H  L  A  R  A  F  G  A  L  E  E  R  H  P  A

38161 CCGCGTCGTGACCGTGACCACCGGTGCCCACGACGTGCTCGGCGACGACCTCGCCCACCC 38220
        R  V  V  T  V  T  T  G  A  H  D  V  L  G  D  D  L  A  H  P

38221 CGAGCACGCCACCGTCCCGGCCGCGGCCAAGGTGATCCCCCGGGAGTACCCGTGGATCGC 38280
        E  H  A  T  V  P  A  A  A  K  V  I  P  R  E  Y  P  W  I  A

38281 CTGCACCGCCCTGGACGTGGAGCCGGGCCTGGACGCCGAGCGGCTGGCGGACCTGATCGT 38340
        C  T  A  L  D  V  E  P  G  L  D  A  E  R  L  A  D  L  I  V

38341 CCGGGAACTCGGCGCGGCGCGCGAGACCACCGTCACCGCCTGCCGCGGCCGACGCCGCTT 38400
        R  E  L  G  A  A  R  E  T  T  V  T  A  C  R  G  R  R  R  F

38401 CACCCCCTGCCCCGTCCGGCAGCCCCTCCCCGCCGCACCGGAACGCCCGGCGGTCCGGCC 38460
        T  P  C  P  V  R  Q  P  L  P  A  A  P  E  R  P  A  V  R  P

38461 CGGCGGCGTCTACCTCGTCTGCGGCGGCCTCGGCGGCATCGGCCTCCACCTCGCCGAGTA 38520
        G  G  V  Y  L  V  C  G  G  L  G  G  I  G  L  H  L  A  E  Y

38521 CCTGGGCCGCGCCCGCACCACCGTCGTCCTCACCCACCGGCGGCCCTTTCCCGCCCCCGG 38580
        L  G  R  A  R  T  T  V  V  L  T  H  R  R  P  F  P  A  P  G

38581 CGCGTGGGACGGGCTGCCCGCGGGACACCCGGAGGCGGCCGTCGTCCGGCGGCTGCGCTC 38640
        A  W  D  G  L  P  A  G  H  P  E  A  A  V  V  R  R  L  R  S

38641 CCTCGCCGCCACCGGCGCCACGGTCGTCGTCCGCCGGGCCGACCTCACCGACCACGACGC 38700
        L  A  A  T  G  A  T  V  V  V  R  R  A  D  L  T  D  H  D  A

38701 GATGCGCGCCCTCGCGGACGAGGTGGAACAGGCCCACGGCCCCGTCCGGGGGGTGGTGCA 38760
        M  R  A  L  A  D  E  V  E  Q  A  H  G  P  V  R  G  V  V  H

38761 CGCGGCCGGGGTGCCCGACACCGCCGGCATGATCCAGCGTCGCGACCGAGCCGGCACGGA 38820
        A  A  G  V  P  D  T  A  G  M  I  Q  R  R  D  R  A  G  T  D

38821 CGCCGCCCTCGCCGCCAAACTGACCGGCACCCTCGTCCTGGACGAGGTGTTCGCCCACCG 38880
        A  A  L  A  A  K  L  T  G  T  L  V  L  D  E  V  F  A  H  R

38881 CGACCTCGACTTCCTCGTCCTGTGCTCCTCGATCGGCACCGTGCTGCACAAGCTGAAGTT 38940
        D  L  D  F  L  V  L  C  S  S  I  G  T  V  L  H  K  L  K  F

38941 CGGCGAGGTCGGCTACGTGGCGGGCAACGAGTTCCTCGACGCCTATGCCGCCCACCGCGC 39000
        G  E  V  G  Y  V  A  G  N  E  F  L  D  A  Y  A  A  H  R  A

39001 GGCCCGCCGCCCCGGCAGAACCCTGTCGATCGCCTGGACCGACTGGCGGGAGTCGGGCAT 39060
        A  R  R  P  G  R  T  L  S  I  A  W  T  D  W  R  E  S  G  M

39061 GTGGGCCGCCGCCCAGCGCCGTCTGACCGAGCGCTACGGCACCGGCGCCGACCTGCCCGT 39120
        W  A  A  A  Q  R  R  L  T  E  R  Y  G  T  G  A  D  L  P  V

39121 ACCGCCCGGGGGCGACCTGCTCGGCGCGATCAGCCCCGAGGAGGGCGTCGACGTCTTCGC 39180
        P  P  G  G  D  L  L  G  A  I  S  P  E  E  G  V  D  V  F  A

39181 CCGGCTGCTCGCCGCCGACACCGGCCCGAACGTCATCGTGTCGGCCCAGGACCTCGACGA 39240
        R  L  L  A  A  D  T  G  P  N  V  I  V  S  A  Q  D  L  D  E

39241 ACTCCTCGCGCGGCACGCGGCGTACACCACCGACGACCACCTCGCCGCCCTCGGCGACCT 39300
        L  L  A  R  H  A  A  Y  T  T  D  D  H  L  A  A  L  G  D  L

39301 GAGGATCGCCGCCGCCCGGGACCGCTCCGCGCCCGCCGCGCCGTACGCGGCCCCCCACAC 39360
        R  I  A  A  A  R  D  R  S  A  P  A  A  P  Y  A  A  P  H  T
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39361 GCCCGCCCAGCGGCGGATCGCCGGCTGGTACCGCGACCTGCTCGGCGTCGAACACGTCGG 39420
      P  A  Q  R  R  I  A  G  W  Y  R  D  L  L  G  V  E  H  V  G

39421 CCTCGACGACGACTTCTTCGCGCTCGGCGGGGACTCGCTGCTCGCCCTGCGCCTGCTGTC 39480
      L  D  D  D  F  F  A  L  G  G  D  S  L  L  A  L  R  L  L  S

39481 GCAGCTGCGGGACGCCTACGGGGTGGAGATCTCCGTCGCCCGCATGTTCGACGAGCCCAC 39540
      Q  L  R  D  A  Y  G  V  E  I  S  V  A  R  M  F  D  E  P  T

39541 GGTGGCGGCGCTGGCCGCCGCCACCGGCCCGCCGCCGGAAGAGACGCCCGGCCAGGAAGA 39600
      V  A  A  L  A  A  A  T  G  P  P  P  E  E  T  P  G  Q  E  E

39601 GGTGGTGCTGTGACCACGCCCCGCATCACCGACCTGCTCACCGAGCTCCGCGGCCGGCAG 39660
      V  V  L  *
                M  T  T  P  R  I  T  D  L  L  T  E  L  R  G  R  Q  (orf23)

39661 GTGACCCTCACGGCCGACGGGGACCGGCTGCACTGCCGCGCGCCCCGGGGCGCGCTCACC 39720
      V  T  L  T  A  D  G  D  R  L  H  C  R  A  P  R  G  A  L  T

39721 GACGAGCTCCTCGCCACCATCCGCGCCCGCCGCGACGAACTCCTCGCCCACCTGCGCGCC 39780
      D  E  L  L  A  T  I  R  A  R  R  D  E  L  L  A  H  L  R  A

39781 GACCGCCGCATCCCGCGCCACGACGGGCCCGCGCCGCTGTCCTTCGCCCAGGAACGGCTC 39840
      D  R  R  I  P  R  H  D  G  P  A  P  L  S  F  A  Q  E  R  L

39841 TGGCTCCTCCACCAGTTCCACCCGCACGACAGCGCCTACAACATCCCCCTGCACATCGCC 39900
      W  L  L  H  Q  F  H  P  H  D  S  A  Y  N  I  P  L  H  I  A

39901 CTGCGCGGGCCCCTGAACCCGGCCGCCCTGCGCGCCGCCCTGGCCGAGGTGGTACGGCGG 39960
      L  R  G  P  L  N  P  A  A  L  R  A  A  L  A  E  V  V  R  R

39961 CACGACGTCCTGCGCACCCGGTACGCCATCAGCCGCGGCCTGCCCCGGCCCGTCGTCGAA 40020
      H  D  V  L  R  T  R  Y  A  I  S  R  G  L  P  R  P  V  V  E

40021 CCGGCCCACACGCCGCCGCTGCCCCTGACCGACCTGACCGGGCTCCCCGCACACCACCGG 40080
      P  A  H  T  P  P  L  P  L  T  D  L  T  G  L  P  A  H  H  R

40081 GACGCCGAACTCGCCCGGCTGGCCGCCCAGGAGGCCAGGCGGCCCTTCGACCTCGCCCAG 40140
      D  A  E  L  A  R  L  A  A  Q  E  A  R  R  P  F  D  L  A  Q

40141 GGCCCGGTGCTGCGGGCCCGGCTCCTCCGAACGGCCCCCGAGGAGCACCGGCTGCTGCTG 40200
      G  P  V  L  R  A  R  L  L  R  T  A  P  E  E  H  R  L  L  L

40201 ACCCGCCATCACATCGCCAGCGACGGCTGGTCGCTCGACATCCTGCTCCGCGAACTGGGC 40260
      T  R  H  H  I  A  S  D  G  W  S  L  D  I  L  L  R  E  L  G

40261 ACGTTCTACCGGGCAGGGCGGGACGGCACACCCGCCGGCCTCGACGCCCTGCCGCTGCGG 40320
      T  F  Y  R  A  G  R  D  G  T  P  A  G  L  D  A  L  P  L  R

40321 TACGCCGACTTCGCCGCGTACCAGCGCGAACAGGCCGAACGGCCGGAGACGGCCGAGCGG 40380
      Y  A  D  F  A  A  Y  Q  R  E  Q  A  E  R  P  E  T  A  E  R

40381 TCGACCCGCTGGGCACGGCACCTGAGGGGCGCCCCCGCGACACTCGACGTCCTCGGGCCC 40440
      S  T  R  W  A  R  H  L  R  G  A  P  A  T  L  D  V  L  G  P

40441 CCGCCCGCCGAACCCTCCCACGCGCCGGCCGGCACCGTACGGACGGACCTTCCCGCCGCC 40500
      P  P  A  E  P  S  H  A  P  A  G  T  V  R  T  D  L  P  A  A

40501 CTCGTCACCGGCCTGCGGCAGCTGGGCGGCCGGGCCCGCACCACGCTCTTCCCGCTCCTG 40560
      L  V  T  G  L  R  Q  L  G  G  R  A  R  T  T  L  F  P  L  L

40561 CTGAGCGCCTTCGGCCTCGCCCTGGCCGGCCCGCCCGGCCCGTACGACGTCATGGTCGGC 40620
      L  S  A  F  G  L  A  L  A  G  P  P  G  P  Y  D  V  M  V  G

40621 ATCCCCGTCGCCGGCCGGCCGCGCACCGAACTGGAGCCGCTCATCGGCTGCTTCGCGACC 40680
      I  P  V  A  G  R  P  R  T  E  L  E  P  L  I  G  C  F  A  T

40681 ATCGCGCCGATGCGGCTGACGAGCGACGGGACCGAGCCGCTGACCCGGCTCGCCGCCCGC 40740
      I  A  P  M  R  L  T  S  D  G  T  E  P  L  T  R  L  A  A  R

40741 GCCCAGCAGCACGTCCAGGACGCGCTGGACGGACCCGACGTCCCCTTCGAGCGGCTCGTG 40800
      A  Q  Q  H  V  Q  D  A  L  D  G  P  D  V  P  F  E  R  L  V
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40801 CACGCGCTGCGTCCGGAGCGGGACCTCGCGGAGAACCCCCTGTTCTCGGCGTCGTTCGCC 40860
      H  A  L  R  P  E  R  D  L  A  E  N  P  L  F  S  A  S  F  A

40861 TTCCAGAACACCCCGCGGACCGCCGTGCGCCTCCCCGGCCTGGACGCCGAGGTGCTGCCC 40920
      F  Q  N  T  P  R  T  A  V  R  L  P  G  L  D  A  E  V  L  P

40921 TCGCCGCCCGTGGCCCCCAAGTTCCCGCTGGCCCTCACCGCGACGGCGCGGGCCGACGGC 40980
      S  P  P  V  A  P  K  F  P  L  A  L  T  A  T  A  R  A  D  G

40981 GGAATGGGCCTGGAGCTGGAGTTCGACCGGGACCGGATCGCCGAGCCGGTCGCGCGGGGG 41040
      G  M  G  L  E  L  E  F  D  R  D  R  I  A  E  P  V  A  R  G

41041 ATCCTCACGTCCTTCCACGCCGCCCTCGCCCGCGCGGTCGCCGACCCCGAGGCCCCGGCG 41100
      I  L  T  S  F  H  A  A  L  A  R  A  V  A  D  P  E  A  P  A

41101 GCGCCCGTACCGGCCGCCGCCGTGGACCGGCGGCCCGGGCGCGAAGGACACGAGTGCCTC 41160
      A  P  V  P  A  A  A  V  D  R  R  P  G  R  E  G  H  E  C  L

41161 CACGAGCCGGTGGCGCGGGCGGCGGCACGCCACCCCGACGCCGTCGCCGTCAGCTGCGGC 41220
      H  E  P  V  A  R  A  A  A  R  H  P  D  A  V  A  V  S  C  G

41221 GGCACCCAGCTCAGCTACGGGGCGCTCGACACCCGCGCCGAACGGCTGGCCGCGGTGCTG 41280
      G  T  Q  L  S  Y  G  A  L  D  T  R  A  E  R  L  A  A  V  L

41281 CGCGCCCACGGCGCCGGCCCCGAGCGGCTGGTGGCCCTGTGCCTGCCCACCGGCCCCGAA 41340
      R  A  H  G  A  G  P  E  R  L  V  A  L  C  L  P  T  G  P  E

41341 TGGGTCGTCGGCGCCCTCGCCATCCTCAAGTCCGGCGCCGCCTACCTGCCGCTCGACCCC 41400
      W  V  V  G  A  L  A  I  L  K  S  G  A  A  Y  L  P  L  D  P

41401 GGCGACCCGGCCGAGCGCCGCGCCTCCGTCGCCGCCGACGCGGGAGCGACGCTGATCGTC 41460
      G  D  P  A  E  R  R  A  S  V  A  A  D  A  G  A  T  L  I  V

41461 TCCGACACCGCGCTTCCCCCGCTCCACCGCGTCGACGTCACGGCCACCCTCCCGGACGGC 41520
      S  D  T  A  L  P  P  L  H  R  V  D  V  T  A  T  L  P  D  G

41521 GCCCCCGAGCCCACCGCCCGGGCCGTCCTGCCCGGCAACCTCGCCTACGCCGTCTACACC 41580
      A  P  E  P  T  A  R  A  V  L  P  G  N  L  A  Y  A  V  Y  T

41581 TCCGGCTCCACCGGCGGCCCCAAGGGCGTGCTCGTCACCCATGCCAACGTCACCGGGCTC 41640
      S  G  S  T  G  G  P  K  G  V  L  V  T  H  A  N  V  T  G  L

41641 CTGGCCGCGTGCCGTGAGGCCCTGCCCGCCCTGGACGCCCCCCGGACCTGGTCGGCGACC 41700
      L  A  A  C  R  E  A  L  P  A  L  D  A  P  R  T  W  S  A  T

41701 CACTCGCCGGCCTTCGACTTCTCCGTCTGGGAGGTCTGGGGCCCGCTGACCGCCGGCGGA 41760
      H  S  P  A  F  D  F  S  V  W  E  V  W  G  P  L  T  A  G  G

41761 CGCCTCGTCCTCGTGCCCCCGGACGTGGCCCGGGCCCCGGACGAACTGTGGGACACCCTC 41820
      R  L  V  L  V  P  P  D  V  A  R  A  P  D  E  L  W  D  T  L

41821 CGCGACGAACAGGTCGAAGTCCTCAGCCAGACCCCCAGCGCGTTCCACCACCTCCTGCCC 41880
      R  D  E  Q  V  E  V  L  S  Q  T  P  S  A  F  H  H  L  L  P

41881 ACCGCCGTGCGCCGGGCGGCCCAGGCCACCGCGCTCGAACTCGTCGTCCTGGGCGGCGAG 41940
      T  A  V  R  R  A  A  Q  A  T  A  L  E  L  V  V  L  G  G  E

41941 GCGTGCGAGCCCGCCCGTCTGACGCCTTGGTGGGACGCCCTGGGCGACCGGCGCCCGGCC 42000
      A  C  E  P  A  R  L  T  P  W  W  D  A  L  G  D  R  R  P  A

42001 GTGGTCAACATGTACGGCATCACCGAGAACACCATCCACGTCACCGTCCGCCGGATGACG 42060
      V  V  N  M  Y  G  I  T  E  N  T  I  H  V  T  V  R  R  M  T

42061 GCGGCGGACCGGTCGGGCAGTCCCGTCGGCCGGCCGCTGCCGGGGCAGCGCGCCGACCTT 42120
      A  A  D  R  S  G  S  P  V  G  R  P  L  P  G  Q  R  A  D  L

42121 CTCGACCCCCACGGCCGGCCCGTCGCGCCGGGCGGGCGGGGCGAACTGTTCGTCGGCGGC 42180
      L  D  P  H  G  R  P  V  A  P  G  G  R  G  E  L  F  V  G  G

42181 GTCGGACTGGCCCGCGGCTACCTCGGCCGGCCCGGCCTCACCGCCCGGAGCTTCCTGCCG 42240
      V  G  L  A  R  G  Y  L  G  R  P  G  L  T  A  R  S  F  L  P

42241 GACGACACCCCCGGCTGGCCGGGCGCGCGCCGCTACCGCTCCGGAGACCTGGCCCGGCTG 42300
      D  D  T  P  G  W  P  G  A  R  R  Y  R  S  G  D  L  A  R  L
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42301 CTGCCCGACGGCGGCCTGGACTACGCGGGCCGCTCCGACGCACAGGTCAAGGTCCGCGGC 42360
      L  P  D  G  G  L  D  Y  A  G  R  S  D  A  Q  V  K  V  R  G

42361 TACCGCGTCGAGCCCGCCGAGACCGAAGCCGCCGCGCTGACCCATCCCGCCGTGCGCCAC 42420
      Y  R  V  E  P  A  E  T  E  A  A  A  L  T  H  P  A  V  R  H

42421 TGCGTGGTCGTGCCACGCGGCGACGGCGACCGGCGCCATCTCGCGGCGTACGTCGTCGCC 42480
      C  V  V  V  P  R  G  D  G  D  R  R  H  L  A  A  Y  V  V  A

42481 GACACCCGCGCCTGCGACGGGCCCGGGCTCCGCACCCACCTGGCCGAGCGGCTGCCCCGC 42540
      D  T  R  A  C  D  G  P  G  L  R  T  H  L  A  E  R  L  P  R

42541 CACCTGGTGCCGGCCTCGGTGGTCTTCCTGAAGCGGATCCCGCTGACCCGCAACGGCAAG 42600
      H  L  V  P  A  S  V  V  F  L  K  R  I  P  L  T  R  N  G  K

42601 CTCGACGTGGCGGCCTTGCCCGACCCGGCCGCCCACCGCGCACCCGCCCGCGAACGCCCG 42660
      L  D  V  A  A  L  P  D  P  A  A  H  R  A  P  A  R  E  R  P

42661 CGCACCGCGACCGAACGGACCCTCACCCGGCTGCTCGCCGCCCTCCTGAAGGCGCCACCG 42720
      R  T  A  T  E  R  T  L  T  R  L  L  A  A  L  L  K  A  P  P

42721 GAGACCATCGGGACGCACGACAACCTCTTCGACCTGGGCGGCGACTCCCTGACGGTCACC 42780
      E  T  I  G  T  H  D  N  L  F  D  L  G  G  D  S  L  T  V  T

42781 CAGTTCCACTCCCGGGTGGTGGAGGAGTTCGCCGTGGACCTCCCGGTGCGCCGGGTCTAC 42840
      Q  F  H  S  R  V  V  E  E  F  A  V  D  L  P  V  R  R  V  Y

42841 CAGGCCCTCGACATCGCGACGCTCGCCGTGACCGTGGACGACTTCCGGCGCCGCGCCGAA 42900
      Q  A  L  D  I  A  T  L  A  V  T  V  D  D  F  R  R  R  A  E

42901 CGCACCGCGGTACTGCGCGCCCTCGCGGCGGCGGAGGCGATGGAACCCGGCGGTACGGCG 42960
      R  T  A  V  L  R  A  L  A  A  A  E  A  M  E  P  G  G  T  A

42961 GGGGAGTCCGGCGGTAATCCGGAGGAGTCCGCCGCTACGGCGCGGGGGCCCGCCGTCGCG 43020
      G  E  S  G  G  N  P  E  E  S  A  A  T  A  R  G  P  A  V  A

43021 GCGAACGAACCCGGCGCTGCGGCGCGTGAGTCCGGCGCCGCGCCGGTGGAGCCCGCCGTC 43080
      A  N  E  P  G  A  A  A  R  E  S  G  A  A  P  V  E  P  A  V

43081 GCAGTACAGGAGTCCGCCGCTACGAAGGGGGAGCCCGGCACCGCAGCGAATGAACTCGGC 43140
      A  V  Q  E  S  A  A  T  K  G  E  P  G  T  A  A  N  E  L  G

43141 GCTGAGGCACGGGAGCCCGGCACCGCAGCGCAGGAACCCGGCACCGACCCCCGGCCACCC 43200
      A  E  A  R  E  P  G  T  A  A  Q  E  P  G  T  D  P  R  P  P

43201 GCCGCCACACCGCAGGACCCCCGCACCACACCGCAGGAAGGACAGCCGTGCCCGCGTCCC 43260
      A  A  T  P  Q  D  P  R  T  T  P  Q  E  G  Q  P  C  P  R  P

43261 GAATGAGCCGGCCGGCCGGCATCGTCGACATCGCGCGCCGTCACGCCGAGCGCACCCCCG 43320
        M  S  R  P  A  G  I  V  D  I  A  R  R  H  A  E  R  T  P  A   (orf22)
      E  *

43321 CCCGTCCCGCGTACGCGTTCCTGCCCGACGGCGAGACGGAGAGCGTCCGCTTCTCCTTCG 43380
        R  P  A  Y  A  F  L  P  D  G  E  T  E  S  V  R  F  S  F  A

43381 CCGACATCGACCGGCGGGCCCGCGCCGTGGCCGCCGTCCTCCAGGACCGCGGCCTGGCCG 43440
        D  I  D  R  R  A  R  A  V  A  A  V  L  Q  D  R  G  L  A  G

43441 GGGAGCGGGTCCTGGTCGCCTATCCCTCCGGGCCCGAGTACGTCCAGGCGTTCCTGGGCT 43500
        E  R  V  L  V  A  Y  P  S  G  P  E  Y  V  Q  A  F  L  G  C

43501 GCCTGTACGCGGGCGTGGTCGCCGTCCCCTGCGACGAGCCGCGCTCCGGCCCGAGCGCGG 43560
        L  Y  A  G  V  V  A  V  P  C  D  E  P  R  S  G  P  S  A  E

43561 AACGGCTCGCCGGGATCCGCGCCGACGCCCGCCCCGCCCTGGCCCTGACCGCCGGCGCCC 43620
        R  L  A  G  I  R  A  D  A  R  P  A  L  A  L  T  A  G  A  P

43621 CCGAGGCCGGGCTCGCCGGCCTGGCCACCCTGGACGTGGCCGGCGTCCCCGACTCCGCCG 43680
        E  A  G  L  A  G  L  A  T  L  D  V  A  G  V  P  D  S  A  A

43681 CCGGGGCCTGGACCGACCCCGTCGCGGGACCGGACGCCCTGGCCTTCCTCCAGTACACCT 43740
        G  A  W  T  D  P  V  A  G  P  D  A  L  A  F  L  Q  Y  T  S
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43741 CCGGATCGACCCGCCGCCCCCGCGGCGTCATGGTCGGCCACGGCAATCTGCTGGCCAACG 43800
        G  S  T  R  R  P  R  G  V  M  V  G  H  G  N  L  L  A  N  E

43801 AGCGCTGCATCGCCGCCGCCTGCGGCCACGACCGGGACTCCACCTTCGTGGGATGGGCGC 43860
        R  C  I  A  A  A  C  G  H  D  R  D  S  T  F  V  G  W  A  P

43861 CGTTCTTCCACGACATGGGCCTGGTCGCCAACCTCCTCCAGCCCCTCTACCTCGGGTCCC 43920
        F  F  H  D  M  G  L  V  A  N  L  L  Q  P  L  Y  L  G  S  L

43921 TGTCGGTGCTGATGCCGCCGATGGCCTTCCTCCAGCGCCCGGCCCGCTGGCTGCGGGCCG 43980
        S  V  L  M  P  P  M  A  F  L  Q  R  P  A  R  W  L  R  A  V

43981 TCTCCCGCTACCGGGCGCACACCAGCGGCGGCCCCAACTTCGCCTACGACCTGTGTGTCG 44040
        S  R  Y  R  A  H  T  S  G  G  P  N  F  A  Y  D  L  C  V  D

44041 ACCGGGTCGGCGAGGACGAGCGGGCCGGACTGGACCTGTCGGGCTGGAAGGTCGCCTACA 44100
        R  V  G  E  D  E  R  A  G  L  D  L  S  G  W  K  V  A  Y  N

44101 ACGGCGCGGAACCTGTACGGGCCGACACCCTGCGACGGTTCACCGACCGCTTCGCCCCCC 44160
        G  A  E  P  V  R  A  D  T  L  R  R  F  T  D  R  F  A  P  H

44161 ACGGCTTCACCCCCGGCGCGCACTTCCCGACCTACGGGCTCGCCGAGGCGACCCTGCTCG 44220
        G  F  T  P  G  A  H  F  P  T  Y  G  L  A  E  A  T  L  L  V

44221 TCGCCACCGGCCCCAAGGGAGTGCCGCCCCGCACCCTGACCGCCGACCGCGCCGCCCTGC 44280
        A  T  G  P  K  G  V  P  P  R  T  L  T  A  D  R  A  A  L  R

44281 GCGCCGGCCGGCTCCGGCCCGCCGGGCCCGGCGAGGCCGGCCTGGAACTGGTCGGCAACG 44340
        A  G  R  L  R  P  A  G  P  G  E  A  G  L  E  L  V  G  N  G

44341 GCACCGCCGGCCTCGACACCACCCTCCGGATCGTCGACCCCGCGACCGCGCGGGAGTGCC 44400
        T  A  G  L  D  T  T  L  R  I  V  D  P  A  T  A  R  E  C  P

44401 CGCCCGGAGAGGTCGGCGAGGTCTGGGTGCGCGGCCCGGGCGTGGCACGCGGCTACTTCG 44460
        P  G  E  V  G  E  V  W  V  R  G  P  G  V  A  R  G  Y  F  G

44461 GCCGCCCGCGCGAGTCCGCGCCGCTGCTCGCCGCCCGCCTGCCCGGCGGCGAAGGACCGT 44520
        R  P  R  E  S  A  P  L  L  A  A  R  L  P  G  G  E  G  P  Y

44521 ACCTGCGGACCGGGGACCTGGGCGCCCTGCACGACGGGGAACTCTTCCTCACCGGACGCC 44580
        L  R  T  G  D  L  G  A  L  H  D  G  E  L  F  L  T  G  R  H

44581 ACAAGGACCTCATCGTCATCCGCGGCCAGAACCACCACCCGCACGACCTCGAACGGACCG 44640
        K  D  L  I  V  I  R  G  Q  N  H  H  P  H  D  L  E  R  T  A

44641 CCGAGCAGGCCCACCCGGCGCTCCGCCCGACCTGCGCCGCCGCGTTCGCGGTGCCCGGGG 44700
        E  Q  A  H  P  A  L  R  P  T  C  A  A  A  F  A  V  P  G  D

44701 ACGGCGCGGAGCGGCTCGTGCTCGTCTGCGAACTCACCTCCTACCGCGCCGTCGACCCGG 44760
        G  A  E  R  L  V  L  V  C  E  L  T  S  Y  R  A  V  D  P  A

44761 CCGCCGTCGCCGAGGCCGTCCGGGCCGCGCTCGCCGCGCGGCACGGCGTCGCCCCGCACA 44820
        A  V  A  E  A  V  R  A  A  L  A  A  R  H  G  V  A  P  H  T

44821 CGCTGGTGGTGCTGCGCCGCGGCGGCATCCCCAAGACCACCAGCGGAAAGGTGCGGCGCG 44880
        L  V  V  L  R  R  G  G  I  P  K  T  T  S  G  K  V  R  R  G

44881 GCCACTGCCGGACGGCCTACCTCGACGGAACGCTCCCCGTTCACACGGCCGTCCGCCTCC 44940
        H  C  R  T  A  Y  L  D  G  T  L  P  V  H  T  A  V  R  L  P

44941 CGGCGGGGGAGGAGGGCACCGAGGCCCTTCCCCTGACCACGGACCCCGGTCGGCTGGCCA 45000
        A  G  E  E  G  T  E  A  L  P  L  T  T  D  P  G  R  L  A  T

45001 CGGCGCTGCGCGACCTGGCCGCCGCCCACGCGGGCCTGGCCGGGCCCCTCCCCGGCACCG 45060
        A  L  R  D  L  A  A  A  H  A  G  L  A  G  P  L  P  G  T  D

45061 ACGAGCCGGTGAGCGCCCTCGGCCTGGACTCGCTCGCCTCCCTGCGGCTCCACCACCACG 45120
        E  P  V  S  A  L  G  L  D  S  L  A  S  L  R  L  H  H  H  V

45121 TCCAGTCCGCCTACGGCGTGACCCTGCCCGTCACCGCCCTGCTCGGCGACACCACTTACC 45180
        Q  S  A  Y  G  V  T  L  P  V  T  A  L  L  G  D  T  T  Y  R

45181 GCCGGCTCGCGGAGCTGACGCTCGCCGCCCCCCGCCCGGCCCGGGCGCCCGAGGGGCAAG 45240
        R  L  A  E  L  T  L  A  A  P  R  P  A  R  A  P  E  G  Q  V
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45241  TCACCGGCGTCTGGCGGCCGTTGACGCACGGGCAGCGCGCCCTGTGGTACGAACAGGCGC  45300
         T  G  V  W  R  P  L  T  H  G  Q  R  A  L  W  Y  E  Q  A  L

45301  TCGCCCCGCACGCGGCCGCCTACCACCTCGTCCGCGCGCTGGCCCTCCGCGGCCCCGTCG  45360
         A  P  H  A  A  A  Y  H  L  V  R  A  L  A  L  R  G  P  V  D

45361  ACGAGGAGGCCCTCGCCGAGGCGGTCCGCCGCGTCGTCCGCCGCCACCCCGCCCTGCGGA  45420
         E  E  A  L  A  E  A  V  R  R  V  V  R  R  H  P  A  L  R  T

45421  CCCGCTTCGCGCTCCGCGACGGCGAACCGGCGCGCCGGACCGAGCCGTACGGACCGGAGC  45480
         R  F  A  L  R  D  G  E  P  A  R  R  T  E  P  Y  G  P  E  L

45481  TGGACGTACGCGACGCCACCGGCCTGCCGGCGGACCGGCTCCGCGAACACCTGGCCGCGG  45540
         D  V  R  D  A  T  G  L  P  A  D  R  L  R  E  H  L  A  A  A

45541  CGGGCGACCGCCCCTTCGACCTGGCCGCCGGCGACAGGCCCGTGAGGCTGACGCTCTACC  45600
         G  D  R  P  F  D  L  A  A  G  D  R  P  V  R  L  T  L  Y  R

45601  GCACGGACGGCGGCCACATCCTGCTGCTGGTCGCCCACCACCTGGTCGCCGACTTCTGGT  45660
         T  D  G  G  H  I  L  L  L  V  A  H  H  L  V  A  D  F  W  S

45661  CCCTCGTCGTCCTCCTGGGCGACCTCGCCCGGGCCCACGCGGGCGAGGACCTGCCGCCCG  45720
         L  V  V  L  L  G  D  L  A  R  A  H  A  G  E  D  L  P  P  A

45721  CGCCGGAGGGGGACCCCGGCGACGAGGCGACGGACGCGGACCGGACGTACTGGCGGCACC  45780
         P  E  G  D  P  G  D  E  A  T  D  A  D  R  T  Y  W  R  H  R

45781  GGCTCGCCGACGCGCCACCCGCCCTCGACCTGCCCACCGACCTCCCCCACCCCGCCGAGC  45840
         L  A  D  A  P  P  A  L  D  L  P  T  D  L  P  H  P  A  E  R

45841  GCGGCTTCGCCGGCGCCACCCACGCCTTCCGGCTGCCCCCGGACCTCACCGCCCGGCTGA  45900
         G  F  A  G  A  T  H  A  F  R  L  P  P  D  L  T  A  R  L  T

45901  CCGCCCTCTCCCGGGAACGGCACTGCACCCTCTTCACCACCCTCCTCGCCGCCCACCAGC  45960
         A  L  S  R  E  R  H  C  T  L  F  T  T  L  L  A  A  H  Q  L

45961  TACTGCTCCACCGCCTGACCGGGCAGGACGACCTCGTCGTGGGCACCCTCCTCGCCCGCC  46020
         L  L  H  R  L  T  G  Q  D  D  L  V  V  G  T  L  L  A  R  R

46021  GCGACACCGCCGAAGCGGCCGGCGCCGTCGGCTACCTGGTCAACCCGCTGCCGCTGCGCT  46080
         D  T  A  E  A  A  G  A  V  G  Y  L  V  N  P  L  P  L  R  S

46081  CCGTACGGGAGCCGGGGGAGACCTTCACGGAACTGCTGCGCCGCACCCGGCGGACCGTGC  46140
         V  R  E  P  G  E  T  F  T  E  L  L  R  R  T  R  R  T  V  L

46141  TGGACGCGGTCGCGCACGGCCGCCACCCCTTCGGGCCGCTCGTCTCCCGTCTCGCCCCCG  46200
         D  A  V  A  H  G  R  H  P  F  G  P  L  V  S  R  L  A  P  A

46201  CGCGCACGCCCGGCCGCGCGCCGCTCCTGCAGAGCCTGTTCGTGCTCCAGCGCGAGTACG  46260
         R  T  P  G  R  A  P  L  L  Q  S  L  F  V  L  Q  R  E  Y  G

46261  GCGACGAGGCGGACGGGTACCGCGCGCTCGCCCTGGGCGTCGGCGGCCGGCTGCGCGTCG  46320
         D  E  A  D  G  Y  R  A  L  A  L  G  V  G  G  R  L  R  V  G

46321  GCGGACTCGACCTGGAGGCACTCGCGTTGCCGCGCCGCTGGTCGCAGCTCGACCTCTCGC  46380
         G  L  D  L  E  A  L  A  L  P  R  R  W  S  Q  L  D  L  S  L

46381  TGAGCATGGCGCGGCTCGGGGACGGGCTGACGGGGGTGTGGGAGTACCGCACCGACCTGT  46440
         S  M  A  R  L  G  D  G  L  T  G  V  W  E  Y  R  T  D  L  F

46441  TCACCGAGGCCACGGTCGCGGAGCTGAGCGAGGCGTTCGTCCACCTGCTGCGGGCGGCCG  46500
         T  E  A  T  V  A  E  L  S  E  A  F  V  H  L  L  R  A  A  V

46501  TCGAGGACCCGGGCGCGCCCGTGGAGACGCTGCCGCTCACCGGCGGCCGGGAGACCGGGC  46560
         E  D  P  G  A  P  V  E  T  L  P  L  T  G  G  R  E  T  G  P

46561  CGCGCCGCGGCCCGTCGGCGGCCCGGCCCGCCCTCCCGCTGCACCGGCTCGTGGCCGCGG  46620
         R  R  G  P  S  A  A  R  P  A  L  P  L  H  R  L  V  A  A  A

46621  CGGCGCGCCGCGATCCCGCACGGACGGCGGTCGTCGCACTCGCCCCGGACGGCACCGCCC  46680
         A  R  R  D  P  A  R  T  A  V  V  A  L  A  P  D  G  T  A  H

46681  ACCACATCAGCCACGGAGCCCTGCACCGCGCGGCCACCACCCTCGCCGCCCGGCTCCGCC  46740
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          H  I  S  H  G  A  L  H  R  A  A  T  T  L  A  A  R  L  R  R

46741 GGGAGGGCGCCGGCCCGGAGCGGCCCGTCGCCGTGCTCGTCGAGCGGGGCCCCTGGCTGC 46800
          E  G  A  G  P  E  R  P  V  A  V  L  V  E  R  G  P  W  L  P

46801 CCGTCGCCTACCTCGGCATCCTGCACGCCGGGGCCACCGTGCTGCCCCTGGACCCGGAGG 46860
          V  A  Y  L  G  I  L  H  A  G  A  T  V  L  P  L  D  P  E  D

46861 ACCCCCCGCACAGGCTCGCCCGGACGATCGCGAACTCGGGGGCGCGGCTGCTGCTCACCG 46920
          P  P  H  R  L  A  R  T  I  A  N  S  G  A  R  L  L  L  T  E

46921 AGACCGGGACCGCCTCGCGCGCGGCCGAGGCGGCCGGTCCCGGCGTACGCGCGCTGACCG 46980
          T  G  T  A  S  R  A  A  E  A  A  G  P  G  V  R  A  L  T  V

46981 TGCGTGAGGGTGCCACCGGCGGCGAGCGGTTCTCGGCGGACGTCCACCCCGAGCAGTCCG 47040
          R  E  G  A  T  G  G  E  R  F  S  A  D  V  H  P  E  Q  S  A

47041 CGTACCTGCTGTACACCTCCGGGTCGACGGGCGACCCCAAGGGCGTGCTCGTCCCGCACC 47100
          Y  L  L  Y  T  S  G  S  T  G  D  P  K  G  V  L  V  P  H  R

47101 GGGCCATCGTCAACCGCCTCCTGTGGATGCAGGAGACCTACCGGCTGCGCCCGGGGGAGC 47160
          A  I  V  N  R  L  L  W  M  Q  E  T  Y  R  L  R  P  G  E  R

47161 GGGTCCTGCACAAGACGCCGGTGACGTTCGACGTCTCGATGTGGGAGCTGCTGTGGCCGC 47220
          V  L  H  K  T  P  V  T  F  D  V  S  M  W  E  L  L  W  P  L

47221 TGACCGCCGGGGCGACCGTCGTCATGGCCCGGCCCGGGACCCACCGCGACCCCGCGCGAC 47280
          T  A  G  A  T  V  V  M  A  R  P  G  T  H  R  D  P  A  R  L

47281 TCGTCCGGCGGATCGCCCGCGAGGCCGTCACCACCGTGCACTTCGTCCCCTCGATGCTCA 47340
          V  R  R  I  A  R  E  A  V  T  T  V  H  F  V  P  S  M  L  T

47341 CCCCGTTCCTCACCGAGCTCGCCCGCGGCACGACGCGGCTGCCCGCGCTGCGGCGCGTGG 47400
          P  F  L  T  E  L  A  R  G  T  T  R  L  P  A  L  R  R  V  V

47401 TGTGCAGCGGGGAAGAGCTGCCCGCGGCCGCGGTGAACCGCGCCGCCGGACTCCTCGACG 47460
          C  S  G  E  E  L  P  A  A  A  V  N  R  A  A  G  L  L  D  A

47461 CCCGGCTGTACAACCTCTACGGCCCGACCGAAGCCGCCGTCGACGTCACCGCCTGGCCCT 47520
          R  L  Y  N  L  Y  G  P  T  E  A  A  V  D  V  T  A  W  P  C

47521 GCCGCCCGCCCGAGCCGGGGCCGGTGCCGATCGGCCTGCCCATCGCCAACACCACCACCG 47580
          R  P  P  E  P  G  P  V  P  I  G  L  P  I  A  N  T  T  T  E

47581 AGGTCCTCGACGGCCGGCTGCGCCCGCTGCCCCGCCCGGTGCCCGGCGAGCTGTACCTGG 47640
          V  L  D  G  R  L  R  P  L  P  R  P  V  P  G  E  L  Y  L  G

47641 GCGGCGCCTGCCTGGCCCATGGCTACCACCACGACCCGGCCCTGACCGCCGCGCGCTTCC 47700
          G  A  C  L  A  H  G  Y  H  H  D  P  A  L  T  A  A  R  F  L

47701 TTCCGGCCCCCGGCGGCGGGCGCCGCTACCGCACCGGGGACCTCGTCCGCCAACGGGCCG 47760
          P  A  P  G  G  G  R  R  Y  R  T  G  D  L  V  R  Q  R  A  D

47761 ACGGGGCACTGGTGTTCCGGGGACGCACGGACGACCAGGTGAAGATCGGCGGCATCCGGG 47820
          G  A  L  V  F  R  G  R  T  D  D  Q  V  K  I  G  G  I  R  V

47821 TCGAGCCCGGCGAGGTGGCGGAGGCGCTTCGGGCCCTGCCCGGCGTCGCCGACGCCGCGG 47880
          E  P  G  E  V  A  E  A  L  R  A  L  P  G  V  A  D  A  A  V

47881 TCGTCCCGCACGACGGGCGGCTGGCGGCGTACGCGGTCGCCGACCCGGTCGGCCCGGCCC 47940
          V  P  H  D  G  R  L  A  A  Y  A  V  A  D  P  V  G  P  A  P

47941 CGGCGGCGGACGCCCTGCGGGACGCGCTGCGCAGGCGGCTGCCCGGCCACCTGGTGCCCG 48000
          A  A  D  A  L  R  D  A  L  R  R  R  L  P  G  H  L  V  P  A

48001 CCGCCCTCACCCTGCTGGACCGGCTGCCCCTCACCCCGGCGGGCAAGCTCGACCGCCGGG 48060
          A  L  T  L  L  D  R  L  P  L  T  P  A  G  K  L  D  R  R  A

48061 CGCTGCCCCACCCGTCGGCCCCGCCCCCGGACGGCGGACGGCCGCCCACGACCGGGACCG 48120
          L  P  H  P  S  A  P  P  P  D  G  G  R  P  P  T  T  G  T  E

48121 AACGGCTCGTCGCCCGGGTGTGGGCCGAACGCCTCGGACGGGAAGTCGTCGGCGTGGACC 48180
          R  L  V  A  R  V  W  A  E  R  L  G  R  E  V  V  G  V  D  R
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48181 GGGACTTCTTCTCCCTGGGCGGCGACTCCGTCCGGGCCCTCGGCGTGACGGCGGCCCTGC 48240
        D  F  F  S  L  G  G  D  S  V  R  A  L  G  V  T  A  A  L  R

48241 GCGCCGCCGGGCTCCCGGTGACGGTCACCGACCTCCTGCGCCTGCCCACCGTGGCCGCCC 48300
        A  A  G  L  P  V  T  V  T  D  L  L  R  L  P  T  V  A  A  L

48301 TCGCCCGCCACGCCGACGAGCGGGCGGATCGCCGACCGGCGCGACAGGAGACGCCCCCCG 48360
        A  R  H  A  D  E  R  A  D  R  R  P  A  R  Q  E  T  P  P  G

48361 GGCCGTTCGCCCTCTGCCCGGAAGCCGCCGGCGTGCCCGGCCTGGAGGACGCCTACCCGA 48420
        P  F  A  L  C  P  E  A  A  G  V  P  G  L  E  D  A  Y  P  M

48421 TGTCGATGGCCCAGCGGGCCGTGCTCTTCCACCGTGACCACAACCCCGGCTACGAGGTCT 48480
        S  M  A  Q  R  A  V  L  F  H  R  D  H  N  P  G  Y  E  V  Y

48481 ACGTCACCAGCGTCGCCGTCTCCACGCCCCTGGACCGCACACGGCTCGCCGCGGCCGTGG 48540
        V  T  S  V  A  V  S  T  P  L  D  R  T  R  L  A  A  A  V  D

48541 ACCGGCTGCTGGACCGGCACGCCTATCTGCGGTCCTCCTTCGACCTCGTGTCCCACCCGG 48600
        R  L  L  D  R  H  A  Y  L  R  S  S  F  D  L  V  S  H  P  E

48601 AGCCCACCCAGCTCGTCTGGACCCACCTGCCCACCCCGCTCGAGGTGGTGGAGTCGTCCG 48660
        P  T  Q  L  V  W  T  H  L  P  T  P  L  E  V  V  E  S  S  D

48661 ACCCCGCCGGTTTCGACGCGTGGCTGCACGCCGAACGCAAGCGCCCCCTCGACGTCGGCA 48720
        P  A  G  F  D  A  W  L  H  A  E  R  K  R  P  L  D  V  G  T

48721 CCGGACCGCTGGCCCGGTTCACCGCGCACGACGCGGGAGCCGCCGGATTCCGGCTGACCG 48780
        G  P  L  A  R  F  T  A  H  D  A  G  A  A  G  F  R  L  T  V

48781 TCAGCAGCTTCGCCCTCGACGGCTGGTGCGTGGCCACCGTGCTCACCGAACTGCTCCGCG 48840
        S  S  F  A  L  D  G  W  C  V  A  T  V  L  T  E  L  L  R  D

48841 ACTACTGGTCCGCGCTGCGCGGCGCGCCCCTCAGCCTCCCGGCACCCGCCGCCTCCTACC 48900
        Y  W  S  A  L  R  G  A  P  L  S  L  P  A  P  A  A  S  Y  R

48901 GCGAGTTCGTCGCCCTCGAACGCGCCGCCCAACACGATCCGGCGCACCGGGAGTTCTGGC 48960
        E  F  V  A  L  E  R  A  A  Q  H  D  P  A  H  R  E  F  W  R

48961 GGACGGAGCTCGCCGGTGCCCGGCCGCATCCGCTGCCCCGCCGCCCGGTGCCACCGCCCG 49020
        T  E  L  A  G  A  R  P  H  P  L  P  R  R  P  V  P  P  P  G

49021 GGCCGGACGGGATCCGCCAGCACCGTCACGTCGTCCCCGTCGAGGACACCGTCGCCAAGG 49080
        P  D  G  I  R  Q  H  R  H  V  V  P  V  E  D  T  V  A  K  G

49081 GCCTGTCGGCGCTCGCCGGCGAGCTGGGTGTCGGGCTCAAACACGTTCTGCTCGGCGTCC 49140
        L  S  A  L  A  G  E  L  G  V  G  L  K  H  V  L  L  G  V  H

49141 ACCTGCGGGTCGTCCGGGCCCTGTCCGGCGACCCCGACGTCATCACGGCCGTGGAGACCC 49200
        L  R  V  V  R  A  L  S  G  D  P  D  V  I  T  A  V  E  T  H

49201 ACGGCCGCCTCGAACGGCACGACGGCGACCGCGTCCTCGGGGTGTTCAACAACATCCTGC 49260
        G  R  L  E  R  H  D  G  D  R  V  L  G  V  F  N  N  I  L  P

49261 CGCTGCGGCAGCGGGTGGACGGCGGGAGCTGGGCCGACCTGGCCCGCGCCGCGCACGCCG 49320
        L  R  Q  R  V  D  G  G  S  W  A  D  L  A  R  A  A  H  A  A

49321 CGGAGGCGCGGACGGGGGAGTACCGCCGCTATCCGCTGGCCCAGGCACAGCGCGACCACG 49380
        E  A  R  T  G  E  Y  R  R  Y  P  L  A  Q  A  Q  R  D  H  G

49381 GCGCGGCCGGGCTCTTCGACACCCTCTTCGTGTTCACCCACTTCCACCTCTACCGCGCGC 49440
        A  A  G  L  F  D  T  L  F  V  F  T  H  F  H  L  Y  R  A  L

49441 TGGCCGACCTGGACGGCATGGCGGTCTCCGACCTGCGGGCCCCCGACCAGACCTACGTAC 49500
        A  D  L  D  G  M  A  V  S  D  L  R  A  P  D  Q  T  Y  V  P

49501 CGCTCACCGCCCACTTCAACGTCGACGCCACGGACGGCGGCGGCCTGCGGCTGCTGCTGG 49560
        L  T  A  H  F  N  V  D  A  T  D  G  G  G  L  R  L  L  L  E

49561 AGTCGGACCCGCGGGAGTTCCCCGACGAGCAGGTCGCGGAGTTCGCCGCGTACTACCGCC 49620
        S  D  P  R  E  F  P  D  E  Q  V  A  E  F  A  A  Y  Y  R  R

49621 GCGCGCTGCGGGCCGCCGCCGACGCCCCGCACCGGCCGTACCGGGACACGCCGTTGACGG 49680
        A  L  R  A  A  A  D  A  P  H  R  P  Y  R  D  T  P  L  T  D
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49681 ACCGGCCGGCCGGTCCGGCGCCGCACCGCGCGGAGCGCTCCGTCCACGCCCTGTTCGCGG 49740
        R  P  A  G  P  A  P  H  R  A  E  R  S  V  H  A  L  F  A  A

49741 CCCCGGCCCGGAACCACCCGGACCGGATCGCGCTCGACGGCGAGGACGGGCCGGTCAGCC 49800
        P  A  R  N  H  P  D  R  I  A  L  D  G  E  D  G  P  V  S  H

49801 ACGGCGCCCTGGCCCGGCGCGCCGCCCGCCTCGCCGGAACGCTGCGGGCCGCGGGCGCCG 49860
        G  A  L  A  R  R  A  A  R  L  A  G  T  L  R  A  A  G  A  G

49861 GGCCGGACACCGTCGTCGGGATCTGGGCGCCGCGCCGCGCCGACGCCGTCGTGGCGCTGC 49920
        P  D  T  V  V  G  I  W  A  P  R  R  A  D  A  V  V  A  L  L

49921 TGGCCGCCCTCCACGCCGGAGCCGCCTACCTGCCCCTGGACCCGGTCCACCCGCCCCGGC 49980
        A  A  L  H  A  G  A  A  Y  L  P  L  D  P  V  H  P  P  R  R

49981 GGCAGCGGCAGGTGCTCACCGAGGCCGGCGCCCGCCTGCTCGTCCTGCCCGCCGGCCTCG 50040
        Q  R  Q  V  L  T  E  A  G  A  R  L  L  V  L  P  A  G  L  D

50041 ACACCCCGCTCCGGGCCTGCGGCCTGCCCGTCGTGGCCCCGGACGACCTCGGCGCGCCCA 50100
        T  P  L  R  A  C  G  L  P  V  V  A  P  D  D  L  G  A  P  I

50101 TCGCCCCCGTGTCCGTCCACCCGGAGCAGCTGGCGGCGGTCATGGCCACGTCCGGCTCCA 50160
        A  P  V  S  V  H  P  E  Q  L  A  A  V  M  A  T  S  G  S  T

50161 CCGGGACGCCCAAGACGATCGGCGTCCCGCAGCGCGCCCTGGCCGGCTACCTCCGCTGGG 50220
        G  T  P  K  T  I  G  V  P  Q  R  A  L  A  G  Y  L  R  W  A

50221 CGATCGGCCACTACCGCCTCGACGAGGAGACCGTCTCCCCGGTGCACTCCTCGCTGGGCT 50280
        I  G  H  Y  R  L  D  E  E  T  V  S  P  V  H  S  S  L  G  F

50281 TCGACCTGACCGTCACCGCGCTGCTCGCACCGCTGGCCGCCGGCGGGCAGGCGCGGCTGA 50340
        D  L  T  V  T  A  L  L  A  P  L  A  A  G  G  Q  A  R  L  T

50341 CCGACTCCGGCGACCCGGGTGCCCTCGGCGCGGCACTGGCCGCCGGCCACCACACCCTGC 50400
        D  S  G  D  P  G  A  L  G  A  A  L  A  A  G  H  H  T  L  L

50401 TCAAGATCACCCCGGCCCATCTGGCCGCCCTCGCCCACCAGTTGGGCGCGCCGACCGCAC 50460
        K  I  T  P  A  H  L  A  A  L  A  H  Q  L  G  A  P  T  A  L

50461 TGCGCACCGTCGTGGCCGGGGGCGAACCCCTGCACGCCGGCCACGTCCGCGCCCTCCGCG 50520
        R  T  V  V  A  G  G  E  P  L  H  A  G  H  V  R  A  L  R  A

50521 CCTTCGCGCCCGGCGCCCGGCTCGTCAACGAGTACGGGCCGACCGAGACCACCGTCGGCT 50580
        F  A  P  G  A  R  L  V  N  E  Y  G  P  T  E  T  T  V  G  C

50581 GCTGTGCCCACGACGTCGCACCGGACCCCGGCGAGGCGCCCATCCCCGTCGGTACCCCGA 50640
        C  A  H  D  V  A  P  D  P  G  E  A  P  I  P  V  G  T  P  I

50641 TCGCGGGCCTCAGCGCGTGCGTCGTCGACGACGCGCTGCCCGCACCGCCCGGCGTGCGGG 50700
        A  G  L  S  A  C  V  V  D  D  A  L  P  A  P  P  G  V  R  G

50701 GCGAGCTGTACATCGGCGGGACGGGCGTCACCCGCGGCTACCTGGGCCGGCCCGCGGCCA 50760
        E  L  Y  I  G  G  T  G  V  T  R  G  Y  L  G  R  P  A  A  T

50761 CCGCCGCCGCCTACGTGCCGGACCCTGCCGCCCCCGGCGCCCGCCGCTACCGCACCGGCG 50820
        A  A  A  Y  V  P  D  P  A  A  P  G  A  R  R  Y  R  T  G  D

50821 ACCTGGCACGCCGGCTGCCGGACGGCACCCTGCTCCTGGCGGGGCGCGCCGACCGCCAGG 50880
        L  A  R  R  L  P  D  G  T  L  L  L  A  G  R  A  D  R  Q  V

50881 TGAAGATCCGCGGCCACCGGGTGGAACCGGGGGAGGTCGAGCAGGTGCTCGGCGGCCACC 50940
        K  I  R  G  H  R  V  E  P  G  E  V  E  Q  V  L  G  G  H  P

50941 CCGGGGTGCGGGAGGCGGCGGTCGTCGCCCACCCGGCACCCGGCGGCGGCCGCCGGCTGG 51000
        G  V  R  E  A  A  V  V  A  H  P  A  P  G  G  G  R  R  L  V

51001 TCGCGTACTGGGTACCGGCCGAACCGGCCCGGCCACCGTCCGCGGACGCGCTCACCGCGC 51060
        A  Y  W  V  P  A  E  P  A  R  P  P  S  A  D  A  L  T  A  L

51061 TGCTCGCCGACCGGCTGCCGCCGTACGCGGTCCCCGCCGAACTCGTCCGCCTGCCCGCCC 51120
        L  A  D  R  L  P  P  Y  A  V  P  A  E  L  V  R  L  P  A  L

51121 TGCCCACCACCCCCAACGGCAAGGTCGACCACACCCGGCTGCCCGCGGCCGGACGGGACC 51180
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          P  T  T  P  N  G  K  V  D  H  T  R  L  P  A  A  G  R  D  R

51181 GGCGACTGGCGGAACTGCTCGACCGGATCGAGGCACTGTCCGACGCCGAGGCGGCCTCGG  51240
        R  L  A  E  L  L  D  R  I  E  A  L  S  D  A  E  A  A  S  A

51241 CACTGCGCGACAGCCGGCCCGCACCCGGGAGTGGCGATGACCGAGCATGACGACCACCCG  51300
        L  R  D  S  R  P  A  P  G  S  G  D  D  R  A  *

51301 CCGGCCCGCCGGGGCCCCGCCGGTTCCGCTGGCCCCGGCGGAAGCCCGCCCGTCCCGCAC  51360

51361 GTGCCGGTGCCCGGGCATGACGACCGCGTCGGACGGCTGCCGGCGGACCGGAGCGTCCCG  51420

51421 CCGACCCGCCGATTCTCTGGGGACCCCGCCGGTTCCGGTGGTGGCCCGCCCGTCCCGCAC  51480

51481 CCGGAGGTGCCGATGCGCGGGCATGACGACCGCGTCGGACGGCTGTCGGCGGACTGGAGC  51540
                    M  R  G  H  D  D  R  V  G  R  L  S  A  D  W  S  (orf21)

51541 GTCCCGCCGACCCGCCTGCCCGCCGGGGACCCGGCCGGTTCCGTCGGCCCCGGCGGAGGC  51600
        V  P  P  T  R  L  P  A  G  D  P  A  G  S  V  G  P  G  G  G

51601 CCGCCCGTCCCGCACGAGGAGGTGACGATGTCGGAGTATGACGACCGCCTCGCGCGGCTG  51660
        P  P  V  P  H  E  E  V  T  M  S  E  Y  D  D  R  L  A  R  L

51661 TCGGACAACCAGCGCGCCCTGCTGGACCGCTGGCTCGCCGAGGACCCCGCCGGCGGTGCC  51720
        S  D  N  Q  R  A  L  L  D  R  W  L  A  E  D  P  A  G  G  A

51721 GGCCCGCTTCGCCCCGACGGCCGCCCGCCCCGCACCGAGGCCGAGCGGATCCTGGCCGGG  51780
        G  P  L  R  P  D  G  R  P  P  R  T  E  A  E  R  I  L  A  G

51781 GTCTGGGAGGAGGTGCTGGAGACCGGCGGGATCGGCGCCGACGACGACTACTTCGCGCTC  51840
        V  W  E  E  V  L  E  T  G  G  I  G  A  D  D  D  Y  F  A  L

51841 GGCGGAGACTCCGTCCACGCCATCGTCATCGTGGCGAAGGCCCGGCAGGCCGGACTCGCC  51900
        G  G  D  S  V  H  A  I  V  I  V  A  K  A  R  Q  A  G  L  A

51901 CTGACCGCCCATGACCTCTTCGAGGCCAGGACCCTCGCGGCCGTGGCGCGGAGAGCCGCC  51960
        L  T  A  H  D  L  F  E  A  R  T  L  A  A  V  A  R  R  A  A

51961 CCGGCCGGCCCCGCCGAGCCCGTCCCCGACGCGGGCGGCGGCGCGGTCCGGTACCCGCTG  52020
        P  A  G  P  A  E  P  V  P  D  A  G  G  G  A  V  R  Y  P  L

52021 ACCCCTATGCAGCAGGGCATGCTCTACCACTCGGCCGGCGGCAGCACGCCCGGCGCCTAC  52080
        T  P  M  Q  Q  G  M  L  Y  H  S  A  G  G  S  T  P  G  A  Y

52081 GTGGTGCAGGTGTGCTGCCGGCTGACGGGGGACCTCGACGTGGCCGCCTTCCGCACCGCC  52140
        V  V  Q  V  C  C  R  L  T  G  D  L  D  V  A  A  F  R  T  A

52141 TGGCAGGCCGTGCTGTCCGCCAACCCGGCGCTGGCCGTCTCCTTCCACTGGTCCGACGGC  52200
        W  Q  A  V  L  S  A  N  P  A  L  A  V  S  F  H  W  S  D  G

52201 TCCCCGCCCGAGCAGGTGGTGGACCCCGACGCGCGCGTCACCGTCGACACGGCCGACTGG  52260
        S  P  P  E  Q  V  V  D  P  D  A  R  V  T  V  D  T  A  D  W

52261 CGGGACCGCACCCCGGCCGAGCGGGACGATGCCTTCGCCCGCTTCCTGGACACCGACCGC  52320
        R  D  R  T  P  A  E  R  D  D  A  F  A  R  F  L  D  T  D  R

52321 GCGGCGGGCTTCGACCTCGCCCGCGCCCCGCTGATGCGGCTGACGCTCTTCCGCGAGGGC  52380
        A  A  G  F  D  L  A  R  A  P  L  M  R  L  T  L  F  R  E  G

52381 GAGCACGCGTACCGCTGCGTGTGGACCCACCACCACCTCGTCCTCGACGGCTGGTCCCAG  52440
        E  H  A  Y  R  C  V  W  T  H  H  H  L  V  L  D  G  W  S  Q

52441 CAGCTCGTCCTGCGCGACGTCCTCGACTGCTACATGCGCCTGCGCGCCGGACGCGGCGCC  52500
        Q  L  V  L  R  D  V  L  D  C  Y  M  R  L  R  A  G  R  G  A

52501 GAGCCGCCCGCCCGGCCGTCCTTCACCGGTCATCTGCGCCGGCTGGAGCGGCAGGACGGG  52560
        E  P  P  A  R  P  S  F  T  G  H  L  R  R  L  E  R  Q  D  G

52561 ATCGACGAGGAGTTCTGGCGCGACCACCTCGGCGGCCTGCCCGCACCCTCCCGCGTCGCC  52620
        I  D  E  E  F  W  R  D  H  L  G  G  L  P  A  P  S  R  V  A

52621 GGTCCCGGCTGCCGCGACGGCCGGGTGGTCGCCGTACGGCGCGCCGAGCACCGGCACCGG  52680
        G  P  G  C  R  D  G  R  V  V  A  V  R  R  A  E  H  R  H  R
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52681 GTCTCCGCGGCGACGGGCCGGGAGCTGACCGGCTTCTGCCGCCGCCACGGGCTGACCCCG 52740
      V  S  A  A  T  G  R  E  L  T  G  F  C  R  R  H  G  L  T  P

52741 GCCGCCGTGCTGCACGGCGGCTGGGCGGTGCTGCTGTCGCTGCACTGCGGCCAGGACGAC 52800
      A  A  V  L  H  G  G  W  A  V  L  L  S  L  H  C  G  Q  D  D

52801 GTGGTCTTCGGCACCACCCTCTCCGGCCGCCCCGAGGACCTGCCCGGCGTGACCGAGTGC 52860
      V  V  F  G  T  T  L  S  G  R  P  E  D  L  P  G  V  T  E  C

52861 GTCGGCCTCTTCATCAACACGCTTCCCCTGCGGGTCCGTTGCGGGGAGGACACGGACGTC 52920
      V  G  L  F  I  N  T  L  P  L  R  V  R  C  G  E  D  T  D  V

52921 GTCGACTGGCTCCACGGCGTCCAAAGCGACCTGGCCGCCCTGTGGGACCACGCGCACGTc 52980
      V  D  W  L  H  G  V  Q  S  D  L  A  A  L  W  D  H  A  H  V

52981 CCGCTCAGCCGCGTcGAGCGCGGTCTCGGACTGGGCCGGgGCGGCGGGCTGTTCGACAGC 53040
      P  L  S  R  V  E  R  G  L  G  L  G  R  G  G  G  L  F  D  S

53041 ATCATGGTCGTCGAGAACTTCCCCGCCGCCGTCGCCGACGGCCACGAGGCGGgCGGGCTG 53100
      I  M  V  V  E  N  F  P  A  A  V  A  D  G  H  E  A  G  G  L

53101 CGGGTGACGGAGCCCCGGGCACTCGTCGACGAGGGCTACCCCCTCGTACTGGAGGCCACC 53160
      R  V  T  E  P  R  A  L  V  D  E  G  Y  P  L  V  L  E  A  T

53161 ACCGGGGACCGGCCGGTGCTGCACGCCCGCTACGACCCCCACCGCCTCGCCGGCGGGCGG 53220
      T  G  D  R  P  V  L  H  A  R  Y  D  P  H  R  L  A  G  G  R

53221 GTCCAGGCGCTGCTCGCCGCCTTCGACGACTACCTCCGGGCGGTGACCGCCGACCCGGCC 53280
      V  Q  A  L  L  A  A  F  D  D  Y  L  R  A  V  T  A  D  P  A

53281 CGCCCGCTGCCGGACCTCCGCGCGGTCCTGGCCCGCGACCACGCGCGCCGGGACGGCGCG 53340
      R  P  L  P  D  L  R  A  V  L  A  R  D  H  A  R  R  D  G  A

53341 GCACGCGGGCGGCGCCGCGCCGCGGACCGCACCCGTCTGACGCTGGCCCGCCGCCGCCCG 53400
      A  R  G  R  R  R  A  A  D  R  T  R  L  T  L  A  R  R  R  P

53401 GCGACGACGACCGAGGGAGAGACACCGTGACATGGACCGTGGTGACCGGAGCCGGCGGCT 53460
      A  T  T  T  E  G  E  T  P  *
                                 M  T  W  T  V  V  T  G  A  G  G  F  (orf20)

53461 TCATCGGCTCCCACCTCGTACGCCGCCTCGTCCGGGACGGGCACCGGGTCCGCGGCGTGG 53520
         I  G  S  H  L  V  R  R  L  V  R  D  G  H  R  V  R  G  V  D

53521 ACCTGGTGCCGCCGCGCTACGGCCCCGGCGAGGCCCAGGAGTTCGTCATCGCCGACCTGC 53580
         L  V  P  P  R  Y  G  P  G  E  A  Q  E  F  V  I  A  D  L  R

53581 GCGACGCGGCGCAGGCCGCGCGGGCCGTCGCCGGCGCGGACTCCGTCTTCGCGCTCGCGG 53640
         D  A  A  Q  A  A  R  A  V  A  G  A  D  S  V  F  A  L  A  A

53641 CCAACATGGGAGGCATCGGCTGGACCCACACCGCGCCCGCCGAGATCCTCCACGACAACC 53700
         N  M  G  G  I  G  W  T  H  T  A  P  A  E  I  L  H  D  N  L

53701 TGCTGATCTCCACCCACACCATCGAGGCATGCCGGGCCGCCGGCGTGCGCACCACCGTCT 53760
         L  I  S  T  H  T  I  E  A  C  R  A  A  G  V  R  T  T  V  Y

53761 ACACCTCCTCGGCCTGCGTCTACCCCGCGTCCCTGCAGCGCGAGCCCGACGCCGCGCCGC 53820
         T  S  S  A  C  V  Y  P  A  S  L  Q  R  E  P  D  A  A  P  L

53821 TGGCCGAGGACCCGGTCTTCCCCGCGGAACCCGACATGGAGTACGGCTGGGAGAAGCTGA 53880
         A  E  D  P  V  F  P  A  E  P  D  M  E  Y  G  W  E  K  L  T

53881 CCACGGAAATCCTGTGCGGCGCCTACCGCCGCAGCCACGGCATGGACATCAAGACAGCCC 53940
         T  E  I  L  C  G  A  Y  R  R  S  H  G  M  D  I  K  T  A  R

53941 GGCTGCACGCCATCTACGGCCCCCTCGGCACGTACACCGGGCCCCGCGCGAAGTCCCTGT 54000
         L  H  A  I  Y  G  P  L  G  T  Y  T  G  P  R  A  K  S  L  S

54001 CGATGCTCTGCGACAAGGTCGCCCGGATACCCGGCGACGAGGGGGAGATAGAGGTCTGGG 54060
         M  L  C  D  K  V  A  R  I  P  G  D  E  G  E  I  E  V  W  G

54061 GGGACGGGACGCAGACCCGCTCCTACTGTTACGTCGACGACTGTGTCGAAGGGCTGATCC 54120
         D  G  T  Q  T  R  S  Y  C  Y  V  D  D  C  V  E  G  L  I  R

54121 GGCTCGCCCGCTCCGACGTGGCGGAACCGGTCAACATCGGCTCCGAGGAGCGCGTCGACA 54180
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          L  A  R  S  D  V  A  E  P  V  N  I  G  S  E  E  R  V  D  I

54181 TCGCGTCGCTCGTCGAGCGGATCGCCGGGGTCGCCGGGAAGAAGGTGCGCTGCGCCTTCG  54240
          A  S  L  V  E  R  I  A  G  V  A  G  K  K  V  R  C  A  F  A

54241 CCCCCGACCGCCCGGTCGGGCCCCGCGGGCGCGTCTCGGACAACACCCGCTGCCGCGAAC  54300
          P  D  R  P  V  G  P  R  G  R  V  S  D  N  T  R  C  R  E  L

54301 TGCTCGGCTGGGCACCGGAGACGTCCCTCGCGGCCGGCCTGGAGCGCACCTACCCGTGGA  54360
          L  G  W  A  P  E  T  S  L  A  A  G  L  E  R  T  Y  P  W  I

54361 TCGAGCGCCAGGTCCTCGCCGAGGCCGGGAGGGCCGATGCCTGAGCACCGCACACCGGTG  54420
                                                                M  (orf19)

          E  R  Q  V  L  A  E  A  G  R  A  D  A  *

54421 AAGGACCTCGGCCGGCTGCTGCTCGGGCACGCCGCGCGCTTCCGGGGCCGCGAGCTGCAG  54480
          K  D  L  G  R  L  L  L  G  H  A  A  R  F  R  G  R  E  L  Q

54481 GACGTCGCCACCCGGGCGCTGCGGGCCTCCGGCGGGGAGAACGCCTGGGTGGTGTCCGTC  54540
          D  V  A  T  R  A  L  R  A  S  G  G  E  N  A  W  V  V  S  V

54541 GTCAACACCAGTCTCCGCGCCCGCCAGGCCGTGGACCACGCGCTGCGGCTCGCCCCCCGC  54600
          V  N  T  S  L  R  A  R  Q  A  V  D  H  A  L  R  L  A  P  R

54601 CGCGGGCTCTCCCGGCTGCGCTACCCGTTCTCCGCCGCCCACCACACGGCCACCCCGCCC  54660
          R  G  L  S  R  L  R  Y  P  F  S  A  A  H  H  T  A  T  P  P

54661 CGGACCCTGTCGCTGCTGTGCCCGACCCGCGAACGCGTCGGCAACGTCGAACGCTTCCTC  54720
          R  T  L  S  L  L  C  P  T  R  E  R  V  G  N  V  E  R  F  L

54721 GACAGCGTCGCCCGCACCGCCGCCGCGCCCGGCCGGATAGAGGCCCTCTTCTACGTCGAC  54780
          D  S  V  A  R  T  A  A  A  P  G  R  I  E  A  L  F  Y  V  D

54781 GACGACGACCCCCAACTCCCTGCCTACCACGAGCTGTTCGAGCACGCCCGGTGGCGCTAC  54840
          D  D  D  P  Q  L  P  A  Y  H  E  L  F  E  H  A  R  W  R  Y

54841 GGACGGATCGGCCGGTGCGCCCTGCACGTCGGCGCCCCCGTCGGCGTACCCCACGCCTGG  54900
          G  R  I  G  R  C  A  L  H  V  G  A  P  V  G  V  P  H  A  W

54901 AACCACCTGGCCCCGGAACGCGGCCGGCGACGTGCTGATGATGGCCAACGACGACCAGCTC  54960
          N  H  L  A  R  N  A  A  G  D  V  L  M  M  A  N  D  D  Q  L

54961 TACATCGACTACGGCTGGGACACCGCCCTCGACGCCCGCGTCACCGAACTGAGCGCCCTG  55020
          Y  I  D  Y  G  W  D  T  A  L  D  A  R  V  T  E  L  S  A  L

55021 CACCCCGACGGCGTCCTGTGCCTGTACTTCGACGACGGCCAGTACCCCGAGGGCGGCTGC  55080
          H  P  D  G  V  L  C  L  Y  F  D  D  G  Q  Y  P  E  G  G  C

55081 GACTTCCCGATGGTGACACGGCCCTGGTACGGCACCCTCGGCTACTTCACCCCGACGATC  55140
          D  F  P  M  V  T  R  P  W  Y  G  T  L  G  Y  F  T  P  T  I

55141 TTCCAGCAGTGGGAGGTCGAGAAGTGGGTCTTCGACATCGCCGACCGGCTGCACCGGCTC  55200
          F  Q  Q  W  E  V  E  K  W  V  F  D  I  A  D  R  L  H  R  L

55201 TACCCCGTCCCCGGCGTCCTCGTCGAACACCGGCACTACCAGGACTACAAGGCACCCTTC  55260
          Y  P  V  P  G  V  L  V  E  H  R  H  Y  Q  D  Y  K  A  P  F

55261 GACGCCACCTACCAGCGGCACCGGATGACACGGGAGAAGTCCTTCGCCGACCACGCCCTG  55320
          D  A  T  Y  Q  R  H  R  M  T  R  E  K  S  F  A  D  H  A  L

55321 TTCCTGCGCACCGAGCCGGACCGCGAGGCGGAGACGGACAGGCTGCGGGCCGTCATCGCC  55380
          F  L  R  T  E  P  D  R  E  A  E  T  D  R  L  R  A  V  I  A

55381 CGGGCAGGGAACACCCCGGACGCCGACCACGCCGACCATGCCGTTCACGACGCGGAGACC  55440
          R  A  G  N  T  P  D  A  D  H  A  D  H  A  V  H  D  A  E  T

55441 TTCTGGTTCACCGGCCTCCTGCGCGAGTCCCACGCCAAGCTGCTCGCGGAACTCGACGAC  55500
          F  W  F  T  G  L  L  R  E  S  H  A  K  L  L  A  E  L  D  D

55501 GCGCCGGGCCCGGCCGCCGGAGCCGTGCTCTTCGCCGACGGCTCCTGGACCGGCGTCGCC  55560
          A  P  G  P  A  A  G  A  V  L  F  A  D  G  S  W  T  G  V  A

55561 TACCGCACCCACCCGCTGGCCACCGCCCTGCTCGCCTCGATCCCCGAGGCCACCCTCGAC  55620
          Y  R  T  H  P  L  A  T  A  L  L  A  S  I  P  E  A  T  L  D
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55621 TCCGGCCGCGCCGACCTCCTCGTCGTCCCGCCCGGCGCGTCCCACCACCACCCCGACGGC 55680
      S  G  R  A  D  L  L  V  V  P  P  G  A  S  H  H  H  P  D  G

55681 ACCGTCGACTCCGCGTTCGGCTCCGACGCCGGCCTCCGCGTCCTGTTCGGACTGCGCGTG 55740
      T  V  D  S  A  F  G  S  D  A  G  L  R  V  L  F  G  L  R  V

55741 CCGGACGCCGCGCAACTCCGCGTCGGCGACGGCCCGGTGCCCTGGGGCAATGGGCAATGC 55800
      P  D  A  A  Q  L  R  V  G  D  G  P  V  P  W  G  N  G  Q  C

55801 CTGATCCACGACACCGCCGCACCGAGCACCCTGCGCAACGACGGCACCGAATCTCTGGCC 55860
      L  I  H  D  T  A  A  P  S  T  L  R  N  D  G  T  E  S  L  A

55861 GCCCTCACCTTCGTGGTGCCGCGCCCGGCACCGGGGGAGTGAGGCCCGTGTGCGGCATCG 55920
      A  L  T  F  V  V  P  R  P  A  P  G  E  *
                                        M  R  P  V  C  G  I  V  (orf18)

55921 TGGCGATCCGCTCCGCCGACGGCGGACTCGACGGCGGTGAACTCACCGCGCCGATGGCCG 55980
        A  I  R  S  A  D  G  G  L  D  G  G  E  L  T  A  P  M  A  D

55981 ACCTGCGCCCGCGCGGCCCCGACGGCGAAGGCACCTGGGTCTCGCCCACCGGCCGGGCCG 56040
      L  R  P  R  G  P  D  G  E  G  T  W  V  S  P  T  G  R  A  A

56041 CCCTCGGCCACACCCGGCTCGCCGTGATCGCCCCCGACGCCGGACGCCAGCCGGTCGCCG 56100
      L  G  H  T  R  L  A  V  I  A  P  D  A  G  R  Q  P  V  A  G

56101 GCCCGGACGGCACCGTCCGGCTCGTCGTCAACGGCGAGTTCTACGGCTACCGGGAGATCC 56160
      P  D  G  T  V  R  L  V  V  N  G  E  F  Y  G  Y  R  E  I  R

56161 GCGCGGAACTGCGCGCCGcCGGCTGCCGGTTCCGCACCGGCAGCGACAGCGAGATCGCCC 56220
      A  E  L  R  A  A  G  C  R  F  R  T  G  S  D  S  E  I  A  L

56221 TCCACCTGTACCTGCGGGACGGCCGGCGGGCACTGGAGCGGCTGCGCGGCGAGTTCGCCT 56280
      H  L  Y  L  R  D  G  R  R  A  L  E  R  L  R  G  E  F  A  F

56281 TCGTCCTCTGGGACGAACGCCGCGCCACCCTCTTCGCCGCCCGCGACCGGTTCGGCGTCA 56340
      V  L  W  D  E  R  R  A  T  L  F  A  A  R  D  R  F  G  V  K

56341 AACCCCTCTACTACACCGAGCGCGACGGGCGGCTCTACGTCGCCTCGACGGTCAGGGCCC 56400
      P  L  Y  Y  T  E  R  D  G  R  L  Y  V  A  S  T  V  R  A  L

56401 TGCTCTCCTGCGGCGCCCCCGCCCGCTGGGACACCGCCGCCTTCGCCGCGCACCTGCAGC 56460
      L  S  C  G  A  P  A  R  W  D  T  A  A  F  A  A  H  L  Q  L

56461 TCGGCCTGCCCCCCGACCGCACCCTCTTCGCCGGCATCCGGCAGCTCCCGCCCGGCTGCC 56520
      G  L  P  P  D  R  T  L  F  A  G  I  R  Q  L  P  P  G  C  H

56521 ACCTCATCGCCGACGCCCACGGCACCCGCGTCACCCCCTACTGGGACCTCGACTACCCGC 56580
      L  I  A  D  A  H  G  T  R  V  T  P  Y  W  D  L  D  Y  P  P

56581 CCGCCGGCGAACTCGCCGCCCGGGGAAGCCTGGACGACCACCTGGACGCGGTACGCGAAC 56640
      A  G  E  L  A  A  R  G  S  L  D  D  H  L  D  A  V  R  E  R

56641 GGACCGACGAGGCCGTACGGTTGCGTACCGTCGCCGACGTGCCCCTCGCCTGCCACCTCA 56700
      T  D  E  A  V  R  L  R  T  V  A  D  V  P  L  A  C  H  L  S

56701 GCGGCGGCCTGGACTCCTCCGCCGTCGCCGCCTCCGCCGCCCGCCACACCCGGCTCACCG 56760
      G  G  L  D  S  S  A  V  A  A  S  A  A  R  H  T  R  L  T  A

56761 CCTTCACCGTCCGCTTCGACGACCCCGCCTTCGACGAGAGCGCCGTCGCCCGGCGCACCG 56820
      F  T  V  R  F  D  D  P  A  F  D  E  S  A  V  A  R  R  T  A

56821 CCGCCCACCTGGCCATCGACCACCGCGAAGTCGCCTCGGAACGCGCCCACTTCGCGGACC 56880
      A  H  L  A  I  D  H  R  E  V  A  S  E  R  A  H  F  A  D  H

56881 ACCTGCGGGACGTCGTCCGCGCCGGCGAGATGGTGCAGGAGAACTCGCACGGCATCGCCC 56940
      L  R  D  V  V  R  A  G  E  M  V  Q  E  N  S  H  G  I  A  R

56941 GGTACCTGCACAGCGCGCACATCAAGAAGGCGGGATTCACCGCCGTCCTCGCCGGGGAGG 57000
      Y  L  H  S  A  H  I  K  K  A  G  F  T  A  V  L  A  G  E  G

57001 GCGGGGACGAACTGTTCCTCGGCTACCCCCAGTTCCGCAAGGACCTGACGCTCAGCCTGT 57060
      G  D  E  L  F  L  G  Y  P  Q  F  R  K  D  L  T  L  S  L  S
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57061 CCGCCGACGCCCGCGACAAGGCCGACCGCGGCTACGCCCGGCTGGTCGCGGCCGGGCTCC 57120
        A  D  A  R  D  K  A  D  R  G  Y  A  R  L  V  A  A  G  L  L

57121 TGCCGCCGTACCTGCGCACCCTCCTCGGCACCCTCGGCTTCCTGCCCTCCTGGATCGTCG 57180
        P  P  Y  L  R  T  L  L  G  T  L  G  F  L  P  S  W  I  V  D

57181 ACCGCCACCTGGCCGTCACCCAGCCCGTCGCCGCCCTGCTCCGCCCCGACTTCGCCGCCG 57240
        R  H  L  A  V  T  Q  P  V  A  A  L  L  R  P  D  F  A  A  E

57241 AACTGGCCCGCGCCGACGCCGCCGCGCCCCTGCTCGCCGCCGGCGCCGGCCTGCTCGCCG 57300
        L  A  R  A  D  A  A  A  P  L  L  A  A  G  A  G  L  L  A  G

57301 GGCGCGCCCCGGCGCACCAGGCCACCTACCTCTTCGCCAAGACCTGGCTGCCCGGCTACC 57360
        R  A  P  A  H  Q  A  T  Y  L  F  A  K  T  W  L  P  G  Y  L

57361 TGCTCGCCGCCGAACGCCTCGACGCGGCCCAGGCCGTCGAGGTGCGGCTGCCCCTCTTCG 57420
        L  A  A  E  R  L  D  A  A  Q  A  V  E  V  R  L  P  L  F  D

57421 ACCACCACCTCTTCGACCTCGTCCGGCACACCCCGCCGGCCTGGTACGACAAGGACGGCA 57480
        H  H  L  F  D  L  V  R  H  T  P  P  A  W  Y  D  K  D  G  T

57481 CCGGCAAGTACCCGCTGCGGGCCGCCATGCGCCACCGGCTGCCGCGCGAGGTGACCGAGG 57540
        G  K  Y  P  L  R  A  A  M  R  H  R  L  P  R  E  V  T  E  G

57541 GCCGCAAACAGGGCTTCCTCGCACCTCCGATGGCCGACGACGACACCCTCCTCGACGCCC 57600
        R  K  Q  G  F  L  A  P  P  M  A  D  D  D  T  L  L  D  A  L

57601 TGCGCGAACGCCTCGCCGGACCGGGCGCGGGCGACGACCCCTTCTTCGACCCGCACGCCG 57660
        R  E  R  L  A  G  P  G  A  G  D  D  P  F  F  D  P  H  A  V

57661 TCCGCGCCCTGCTGGACCGGCTGGCCGCCGCACCCCCGGGGCAGCGGTCCGGCGGCGAGA 57720
        R  A  L  L  D  R  L  A  A  A  P  P  G  Q  R  S  G  G  E  K

57721 AACTCCTCCAACTCGTCGCGAGCACCGCCGAACTGGCCGACGAGTTCGGCCTCACCACCG 57780
        L  L  Q  L  V  A  S  T  A  E  L  A  D  E  F  G  L  T  T  A

57781 CCCCCAGCGGGCAGAAAGGCGGCAACGGTGGCTGACCTCGATCCCGGCACGCTCTCCGAG 57840
        P  S  G  Q  K  G  G  N  G  G  *

57841 GCCGAGCTGACCGCCCGGATCGCCGCCCTGTCCCCCGAACGCCGGGCGGCGTTCGAGAAG 57900

57901 ATGCTGCACGGCGCCGCGCACCCCCGCCCCGGCATCCCGCGCCGCGGCGCCACCGCGGCA 57960
        M  L  H  G  A  A  H  P  R  P  G  I  P  R  R  G  A  T  A  A   (orf17)

57961 CCGGCCTCCTACGGCCAGGAACGCCTGTGGCTGCTCACCGGGCTGCTGCCCACCGCCTAC 58020
        P  A  S  Y  G  Q  E  R  L  W  L  L  T  G  L  L  P  T  A  Y

58021 AACTACGCCACCGCCCTGCGGCTGCGCGGCGACCTGTCCGTCCCCGCGCTGCGCGGCGCC 58080
        N  Y  A  T  A  L  R  L  R  G  D  L  S  V  P  A  L  R  G  A

58081 CTGCGCGGCATCGTCCGCCGCCACGAGGTGCTGCGCACCACCTTCCGGCTGGACGGCGAC 58140
        L  R  G  I  V  R  R  H  E  V  L  R  T  T  F  R  L  D  G  D

58141 GACCTCATCCAGGTCGTCCACCCCACGGCGGACGTCCCCGTGCGCCTGGCCGACCTCACC 58200
        D  L  I  Q  V  V  H  P  T  A  D  V  P  V  R  L  A  D  L  T

58201 GGACGCTCCGCCGACACCGGGCGGCTGATGCGCGAGGAGGCCCGCCGCCCCTTCGACCTG 58260
        G  R  S  A  D  T  G  R  L  M  R  E  E  A  R  R  P  F  D  L

58261 GAGCACGGGCCGCTGCTGCGGCTGACCCTCTTCCGGCTCGGCCCCCGCGACCACCTCGCC 58320
        E  H  G  P  L  L  R  L  T  L  F  R  L  G  P  R  D  H  L  A

58321 CTGCTGGCCGTCCACCACGCCGTCACCGACGGCTGGTCCAACGGCGTCCTCGTGACCGAA 58380
        L  L  A  V  H  H  A  V  T  D  G  W  S  N  G  V  L  V  T  E

58381 CTCGCCACCGGCTACCGGGAACTGCGCGCCGGACGCCCCGACCGGCGGCCCGCCCCGCCG 58440
        L  A  T  G  Y  R  E  L  R  A  G  R  P  D  R  R  P  A  P  P

58441 GTCCAGTACGGCGACTACGCGCACTGGCAGCGCGAGCGGCTGACCGGGCCCGAACTGCGG 58500
        V  Q  Y  G  D  Y  A  H  W  Q  R  E  R  L  T  G  P  E  L  R

58501 GCCCTGGAGGACTACTGGCGCACCGCCGTACGCGACCTGCCCAGGACGGACCTGCCCACC 58560
        A  L  E  D  Y  W  R  T  A  V  R  D  L  P  R  T  D  L  P  T
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58561 GACCGCCCCCGCCCCGCCGCCGGCGCGGCGAGGGCGCCAACCACGCCCTGCTGCTCTCG 58620
      D  R  P  R  P  A  A  R  R  G  E  G  A  N  H  A  L  L  L  S

58621 CCGGAGCTGACCGGCCGGCTCGCCGACCTGCGCCGCCGCGAGGGCGGGTCGCTGTTCATG 58680
      P  E  L  T  G  R  L  A  D  L  R  R  R  E  G  G  S  L  F  M

58681 CTCGTGCTCTCCGCGCTCCTGGTCGTCCTGCGTGGCACCGGCGGCCGGGACCGGCTCGCC 58740
      L  V  L  S  A  L  L  V  V  L  R  G  T  G  G  R  D  R  L  A

58741 GTCGGCACCCTCGTCGCCGGCCGCACCCGCCCCGAACTCGAGCCGCTCATCGGCTACTTC 58800
      V  G  T  L  V  A  G  R  T  R  P  E  L  E  P  L  I  G  Y  F

58801 GTCAACGTCCTGCTGCTGCCCTTCGAGACCGGCGGCCGGACCTCCTTCGCCGAGCTGTGG 58860
      V  N  V  L  L  L  P  F  E  T  G  G  R  T  S  F  A  E  L  W

58861 CGGCGGGTCCGCGGCCGGCTGGTGGAGGCGTACGCCCACCAGGAACTGCCGCTGGAGAAG 58920
      R  R  V  R  G  R  L  V  E  A  Y  A  H  Q  E  L  P  L  E  K

58921 GCCCTGGAGCTGCTGCGCGCCGACGGCACCGCCCCCGCCGACCCGCCGGTCGGCGTGGTC 58980
      A  L  E  L  L  R  A  D  G  T  A  P  A  D  P  P  V  G  V  V

58981 TGCGTCGCCCAGCAGCCCGCCCCCGCGATCACCCTGCCCGGACTCGACGCGAGCGTCGAG 59040
      C  V  A  Q  Q  P  A  P  A  I  T  L  P  G  L  D  A  S  V  E

59041 GACGTCGACCTGGGCACCGCCCAGTTCGACCTCGTCGTCGAGGTGCGCGAACGGCCGGAA 59100
      D  V  D  L  G  T  A  Q  F  D  L  V  V  E  V  R  E  R  P  E

59101 GGCGTGCAGATCGCCTTCCAGTACGACCGGGACCTGTTCGACGCGGCCACGGTCCGGCTC 59160
      G  V  Q  I  A  F  Q  Y  D  R  D  L  F  D  A  A  T  V  R  L

59161 CTCGCCGACCACGTGCACGCCGTCCTCGACCAGGCCGCCGCCGACCCCACCCTGCCCTGT 59220
      L  A  D  H  V  H  A  V  L  D  Q  A  A  A  D  P  T  L  P  C

59221 GCCGAGCTGCCCGCCCCGCCGGCCCCCGCGGCCCCGGCCCGCACGGCCGGCGCCACGACG 59280
      A  E  L  P  A  P  P  A  P  A  A  P  A  R  T  A  G  A  T  T

59281 CTGCACGCCCTGTTCGAGTCCCGCGCCGCGAAGAGCCCCGACGCGGTCGCCCTCGTCGAC 59340
      L  H  A  L  F  E  S  R  A  A  K  S  P  D  A  V  A  L  V  D

59341 GGCGGCCACCGCGTCACCTACCGGACCCTCAACACCCGCGCCAACCGGCTCGCCCGCCAC 59400
      G  G  H  R  V  T  Y  R  T  L  N  T  R  A  N  R  L  A  R  H

59401 CTGCGCGCGGTCGGCGTGCGTACCGAGGACCGGGTGGCGCTGCGCCTGCCCCGCGGCACC 59460
      L  R  A  V  G  V  R  T  E  D  R  V  A  L  R  L  P  R  G  T

59461 GACGCGGTGACCGCCACCCTCGCCGCCCTCAAGGCCGGCGCCGCGTACGTACCCCTCGAC 59520
      D  A  V  T  A  T  L  A  A  L  K  A  G  A  A  Y  V  P  L  D

59521 CCCGCCCTCCCCGAGGAACGGCTGACCCGCGTCCTCGCCGACGCCCGCCCCGCCGTGGTC 59580
      P  A  L  P  E  E  R  L  T  R  V  L  A  D  A  R  P  A  V  V

59581 CTCACCCCCGCGTATCTGCACGACCGGTCCGCCGAGATCACCGCCCACGCCGGCCATGAC 59640
      L  T  P  A  Y  L  H  D  R  S  A  E  I  T  A  H  A  G  H  D

59641 CTCAACCTCCCCGTCCACCCCGACAACCTCGCCTACCTCCTCCACACCTCCGGATCCACC 59700
      L  N  L  P  V  H  P  D  N  L  A  Y  L  L  H  T  S  G  S  T

59701 GGCACCCCCaAGGGCGTCCTCGGCACcCCACCGGGGCGCGGTCAACCGCGTCGACTGGATG 59760
      G  T  P  K  G  V  L  G  T  H  R  G  A  V  N  R  V  D  W  M

59761 AGCACCGCGTACCCGTTCCGGACCGGCGACGTGGCCGTCGCCCGCACCGCGCCCGGCTTC 59820
      S  T  A  Y  P  F  R  T  G  D  V  A  V  A  R  T  A  P  G  F

59821 GTCGACGCGGTCTGGGAACTCTTCGGCCCCCTGGCCGCCGGCGTCCCCCTCGTCCTCCTG 59880
      V  D  A  V  W  E  L  F  G  P  L  A  A  G  V  P  L  V  L  L

59881 CCGACCGACGAGGCGCGCGACCCGGCCCTGCTGACGGCGGCGCTGGAACGGCACCGGGTG 59940
      P  T  D  E  A  R  D  P  A  L  L  T  A  A  L  E  R  H  R  V

59941 AGCCGGATGGTGACGGTCCCGTCGCTGCTGACCATGCTCCTGGACGAGTCCGCCCGCGCG 60000
      S  R  M  V  T  V  P  S  L  L  T  M  L  L  D  E  S  A  R  A

60001 ACGGACCTCGGCACCCGCCTGGCCTGCCTCCGCACCTGGATCACCAGCGGCGAGCCCCTG 60060
      T  D  L  G  T  R  L  A  C  L  R  T  W  I  T  S  G  E  P  L
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60061  CCGCCCGCGCTCGCCCGGCGGTTCCACGACCGCCTGCCCGGCCGCACCCTGCTGAACCTG  60120
       P  P  A  L  A  R  R  F  H  D  R  L  P  G  R  T  L  L  N  L

60121  TACGGCTCCTCCGAGACCGCCGCCGACGCCACCGCGGCCCGCATCGACCCGGCGCCCGGG  60180
       Y  G  S  S  E  T  A  A  D  A  T  A  A  R  I  D  P  A  P  G

60181  ACTGCGCTCCCGGAGCGGTCCCCGATCGGCACGCCCATCACCGGCGTCAGCGCCCTCGTC  60240
       T  A  L  P  E  R  S  P  I  G  T  P  I  T  G  V  S  A  L  V

60241  CGCGGCCCGGACCTGCGCCCGCTGCCCGCGCTGATGCCCGGCGAGCTGTACGCCGGGGGC  60300
       R  G  P  D  L  R  P  L  P  A  L  M  P  G  E  L  Y  A  G  G

60301  GCGTGCGTGGCCCGCGGCTACCACGCCCGTCCGGCCGAGACCGCCGCGGCGTTCCCGCCG  60360
       A  C  V  A  R  G  Y  H  A  R  P  A  E  T  A  A  A  F  P  P

60361  GATCCCGACGGCGGGCCCGGCGCCCGGATGTTCCGTACCGGTGACAGGGCCCGGCTGCGG  60420
       D  P  D  G  G  P  G  A  R  M  F  R  T  G  D  R  A  R  L  R

60421  GCCGACGGCCGGCTGGAACTCCTGGGGCGCGTGGACCGGCAGGTGCAGATCCGCGGCCAG  60480
       A  D  G  R  L  E  L  L  G  R  V  D  R  Q  V  Q  I  R  G  Q

60481  CGCGCCGAGCCCGGCGAGGTCGAACACGCCCTGCTGGCCCACCCGGCCGTACGGGCCGCC  60540
       R  A  E  P  G  E  V  E  H  A  L  L  A  H  P  A  V  R  A  A

60541  GCCGTCACGGCGAACCCCGACGCCACCGGCCTGTGGGCGTACGTGCGGCTCGCTCCCGGC  60600
       A  V  T  A  N  P  D  A  T  G  L  W  A  Y  V  R  L  A  P  G

60601  CCGTTCGCCGCCGGCTCCCCCCAGACCGAGCTGACCGCCTTCCTGCGCCGCACGCTCCCT  60660
       P  F  A  A  G  S  P  Q  T  E  L  T  A  F  L  R  R  T  L  P

60661  GCCCACCTCGTGCCCACCGCCGTCACCGTCCTGGACGAGCTGCCGGTGACCGCGCACGGC  60720
       A  H  L  V  P  T  A  V  T  V  L  D  E  L  P  V  T  A  H  G

60721  AAGACCGACCACGCGCGGCTGCCCGCCCCCGACCCCCGGGCCGGGCGCCCCGCCCCGACC  60780
       K  T  D  H  A  R  L  P  A  P  D  P  R  A  G  R  P  A  P  T

60781  GCCCCCCGCACCCCCACCGAGCGTACGGTCGCCGACGTCTTCGCCGGGGTGCTCGGCCTG  60840
       A  P  R  T  P  T  E  R  T  V  A  D  V  F  A  G  V  L  G  L

60841  GAGGGGCCGGTCGGCGCGCACGACGACTTCTTCCTCCTCGGCGGGCACTCCCTCCTCGCC  60900
       E  G  P  V  G  A  H  D  D  F  F  L  L  G  G  H  S  L  L  A

60901  GCCCGCAGTCGCGGCGGAACTCCGCGCCCGCCGCGGCGTCCGGATCGGGCTGAGCGACGT  60960
       A  R  S  R  G  G  T  P  R  P  P  P  R  R  P  D  R  A  E  R  R

60961  CTTCGCGGCCCCCACCGTCGCCGCAGCGTCGCCGCCCGGACCGACGCCGCCCGGCCCGGC  61020
       L  R  G  P  H  R  R  R  S  V  A  A  R  T  D  A  A  R  P  G

61021  ACCGGCCCCGAGCACACCCCGTTCGTCACCGACCCCGGCGCCCGGCACGAGCCGTTCCCG  61080
       T  G  P  E  H  T  P  F  V  T  D  P  G  A  R  H  E  P  F  P

61081  CTCACCGACGTCCAGCGGGCCTACTACGTGGGACGCGAGGGCGGGTTCGCCCTCGGCGGC  61140
       L  T  D  V  Q  R  A  Y  Y  V  G  R  E  G  G  F  A  L  G  G

61141  GTCTCCACCCACGCCTACCTGGAGATCGAGGCCCCGCGGATCGACGTCGCACGGTTTACC  61200
       V  S  T  H  A  Y  L  E  I  E  A  P  R  I  D  V  A  R  F  T

61201  GGCGCGCTGCGCGGGGTGATCGCCCGGCACCCCATGCTGCGCGCCGTGATCCGTCCCGAC  61260
       G  A  L  R  G  V  I  A  R  H  P  M  L  R  A  V  I  R  P  D

61261  GGGCTCCAGCAGGTGCTCACCGACGTCCCCCCGTACGACGTGGCCGTGCACGACCTGCGC  61320
       G  L  Q  Q  V  L  T  D  V  P  P  Y  D  V  A  V  H  D  L  R

61321  GACCTGGACGAGCCCGCGCGGCAGCGCCGACGCGCCGCGCTGCGCGAGGAGATGTCCCAC  61380
       D  L  D  E  P  A  R  Q  R  R  R  A  A  L  R  E  E  M  S  H

61381  CAGGTGGTGCCCGCCGACCTCTGGCCCCTGTTCGACGTCCGCGTCTCCCTCGGCCCCACG  61440
       Q  V  V  P  A  D  L  W  P  L  F  D  V  R  V  S  L  G  P  T

61441  GACGCCCTCGTCCACGTGGGGGTGGACGCGCTGATCTGCGACGCCCACAGCTTCGGCCTC  61500
       D  A  L  V  H  V  G  V  D  A  L  I  C  D  A  H  S  F  G  L

61501  GTCCTGGCCGAACTCGCGGCCCGTTACGCCGACCCCGCACGCCGCTTCCCGCCCCTGACG  61560
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          V  L  A  E  L  A  A  R  Y  A  D  P  A  R  R  F  P  P  L  T
61561 GCGGACTTCCGGGACCACGTCCTCCATCAGGAGGCGCTCCGCGGAACCGCCGAGTACGCG 61620
      A  D  F  R  D  H  V  L  H  Q  E  A  L  R  G  T  A  E  Y  A
61621 GCGGCGGAGCGGTACTGGCGCGAACGCCTGCCCGAGCTGCCGCCCGGCCCCGAACTGCCC 61680
      A  A  E  R  Y  W  R  E  R  L  P  E  L  P  P  G  P  E  L  P
61681 CTGGCCGTCGCGCCCGAGACCCTCGGCACCCCGCGCTTCACCCGCCGCTCCGGCCGGCTG 61740
      L  A  V  A  P  E  T  L  G  T  P  R  F  T  R  R  S  G  R  L
61741 GACGCGGCCTCCTGGACGGCGGTCAAGGACCGGGCCCGCCGCGCCGGGCTCAGCCCCTCC 61800
      D  A  A  S  W  T  A  V  K  D  R  A  R  R  A  G  L  S  P  S
61801 GGCGTACTGCTGGCGGCGTTCGCCGAGGTGATCACCGCGTGGAGCGGCCGGCCGCGCTAC 61860
      G  V  L  L  A  A  F  A  E  V  I  T  A  W  S  G  R  P  R  Y
61861 TCGCTGATGCTGACGGTCTTCGACCGCCCGCCGCTCCACCCGGACCTCGGGCGGATCGTC 61920
      S  L  M  L  T  V  F  D  R  P  P  L  H  P  D  L  G  R  I  V
61921 GGCGACTTCACCTCGCTCAGCCTGCTGGAGGTCGACCACAGTCGGCCCGGCGACTTCACC 61980
      G  D  F  T  S  L  S  L  L  E  V  D  H  S  R  P  G  D  F  T
61981 GACAGGGCCCGCGCCCTCCAGCGCCGCCTGTGGCAGGACCTCGACCACCTGGCGGTCGGC 62040
      D  R  A  R  A  L  Q  R  R  L  W  Q  D  L  D  H  L  A  V  G
62041 GGCGTGACGGTGACACGGGAACGGGCGCTGCGCCACGACGCCCGACCCGGTCTGCTCACA 62100
      G  V  T  V  T  R  E  R  A  L  R  H  D  A  R  P  G  L  L  T
62101 CCCGTCGTCTTCACCTCCGACCTGCCTGTCGGCGAGACCGCGGCCGAGGACGCGGACGGG 62160
      P  V  V  F  T  S  D  L  P  V  G  E  T  A  A  E  D  A  D  G
62161 GGAGAGGGATGGGCGCTCGGAGAGCCCGTCTACGGCGTCAGCCAGACCCCGCAGGTCCAT 62220
      G  E  G  W  A  L  G  E  P  V  Y  G  V  S  Q  T  P  Q  V  H
62221 CTCGACCATCAAGTCGCCGAAGACCGAGGGGAGTTGGTCTTCAACTGGGACGCCGTGGAA 62280
      L  D  H  Q  V  A  E  D  R  G  E  L  V  F  N  W  D  A  V  E
62281 GACCTGTTCGCCCCGGGCGCCCTGGACGCCATGTTCGCCGCCTACACCGCCTCGCTGACC 62340
      D  L  F  A  P  G  A  L  D  A  M  F  A  A  Y  T  A  S  L  T
62341 CGCCTGGCCCGGAGCCCCGAAGCCTGGCGGCGGCCCGGCACGCCGCCGCTGCCCACCGCC 62400
      R  L  A  R  S  P  E  A  W  R  R  P  G  T  P  P  L  P  T  A
62401 CAGGCGGCCGTGCGCCGGCGCACCGCCGCGACCGAGGCGCCCCTGCCCGCCCGCCTGCTG 62460
      Q  A  A  V  R  R  R  T  A  A  T  E  A  P  L  P  A  R  L  L
62461 CACGAGGCCGTCGGCGACGCGGCCCGGCGCCACGCCGACCTGACCGCCCTGGTCGACGGC 62520
      H  E  A  V  G  D  A  A  R  R  H  A  D  L  T  A  L  V  D  G
62521 GACACCCGGATGACCTACCGGCGACTGACCGAGCACGCCCGGCGCGTCGGCCGCACGCTG 62580
      D  T  R  M  T  Y  R  R  L  T  E  H  A  R  R  V  G  R  T  L
62581 CGCCGCCTCGGCGCCCGCCCCGGCCGCCTGGTCCCGGTGGTCGCCCGCAAGGGGTGGCGG 62640
      R  R  L  G  A  R  P  G  R  L  V  P  V  V  A  R  K  G  W  R
62641 CAGGCCGTCGCCGCGCTGGGCGTCCTGGAGTCGGGGGCGGCGTACCTGCCCCTGGACCCC 62700
      Q  A  V  A  A  L  G  V  L  E  S  G  A  A  Y  L  P  L  D  P
62701 GAACTGCCCGCCGAACGGCTCGTCCACCTCGTACGGCGCGCCGAAGCCGCCCTCCTCCTC 62760
      E  L  P  A  E  R  L  V  H  L  V  R  R  A  E  A  A  L  L  L
62761 ACCGAACGCGCCCTGCTGGACACGCTCGCCGTCCCCGTCGGCGTCACCGTGCTCGCGGTG 62820
      T  E  R  A  L  L  D  T  L  A  V  P  V  G  V  T  V  L  A  V
62821 GACGACGACGCGGCCCTCGACGCCGACGGCGGCCCGCTGCAGAGCGTGCAGAACCTCACC 62880
      D  D  D  A  A  L  D  A  D  G  G  P  L  Q  S  V  Q  N  L  T
62881 GACCTGGCGTACACCATCTTCACCTCGGGCTCCACCGGCGAACCCAAGGGCGTCATGATC 62940
      D  L  A  Y  T  I  F  T  S  G  S  T  G  E  P  K  G  V  M  I
62941 GACCACCTCGGCGCGGCCAACACCCTGGAATGCGTCAACCGCCGCTTCGGCACCGGCCCC 63000
      D  H  L  G  A  A  N  T  L  E  C  V  N  R  R  F  G  T  G  P
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63001 GGCGACGCGGTCCTCGCCGTCTCCTCCCCGAGCTTCGACCTCGCCGTCTACGACCTGTTC 63060
      G  D  A  V  L  A  V  S  S  P  S  F  D  L  A  V  Y  D  L  F

63061 GGCGTGCTGGCCGCCGGCGGCACCGTGGTCGTCCCCGCCCACGACCGCCGGCGCGACCCC 63120
      G  V  L  A  A  G  G  T  V  V  V  P  A  H  D  R  R  R  D  P

63121 GGACACTGGGCCGAGCTGATCCGGCGCGAGCGGGTCACCCTGTGGAACTCCGTCCCCGCG 63180
      G  H  W  A  E  L  I  R  R  E  R  V  T  L  W  N  S  V  P  A

63181 CTGGGCACCCTGCTCACCGAGTACGCCGAGGCCCTCGCCCCCGACGCCCTGCGCACCCTG 63240
      L  G  T  L  L  T  E  Y  A  E  A  L  A  P  D  A  L  R  T  L

63241 CGGGCGGTGCTCCTCAGCGGCGACTGGATCCccctcggactgcccgaccGGATCCGCGCC 63300
      R  A  V  L  L  S  G  D  W  I  P  L  G  L  P  D  R  I  R  A

63301 CTGTCCGCCCCCGGCGCCACCGTGATGAGCCTCGGCGGCGCGACCGAAGCCTCCATCTGG 63360
      L  S  A  P  G  A  T  V  M  S  L  G  G  A  T  E  A  S  I  W

63361 TCGGTCTGGTACGAGATCGGGAAGGTGCACGAGGCGTGGAGCAGCATCCCCTACGGCACC 63420
      S  V  W  Y  E  I  G  K  V  H  E  A  W  S  S  I  P  Y  G  T

63421 CCCATGGCCAACCAGCGGCTGGAGGTCCTCGACGAGCAGCTGCGGCCCCGGCCCGACTGG 63480
      P  M  A  N  Q  R  L  E  V  L  D  E  Q  L  R  P  R  P  D  W

63481 GTGCCCGGCGAGCTGTACATCGGCGGCACCGGCGTCGCCAAGGGCTACTGGCGCGACCCG 63540
      V  P  G  E  L  Y  I  G  G  T  G  V  A  K  G  Y  W  R  D  P

63541 GAACAGACCTCCCTGCGCTTCCCCGTCCACCCGGGCAGCGGGCAACGCCTGTACCGCACC 63600
      E  Q  T  S  L  R  F  P  V  H  P  G  S  G  Q  R  L  Y  R  T

63601 GGGGACTTCGCCCGCCACCTCCCCGACGGCACGCTGGAATTCCTGGGCCGGCAGGACGAC 63660
      G  D  F  A  R  H  L  P  D  G  T  L  E  F  L  G  R  Q  D  D

63661 CAGGTGAAGATCGGCGGATTCCGGGTCGAACTGGGCGAGGTCGAGGCGGCCCTCGGCCGA 63720
      Q  V  K  I  G  G  F  R  V  E  L  G  E  V  E  A  A  L  G  R

63721 CTGCCCGACGTCGCCGCCGGCGCGGTGATCGCCACCGGTGACCCGCGGGGCGACCGCCGC 63780
      L  P  D  V  A  A  G  A  V  I  A  T  G  D  P  R  G  D  R  R

63781 CTCGTCGGCTTCGCCGTACCGGCCCGGGAGGGCGGCTTCGACGCGGCCGGGCTCCGACGG 63840
      L  V  G  F  A  V  P  A  R  E  G  G  F  D  A  A  G  L  R  R

63841 CAACTCGCCCGGCGGCTGCCCGCCTACATGGTCCCCACGACCCTGCTGCCCCTGGACCGG 63900
      Q  L  A  R  R  L  P  A  Y  M  V  P  T  T  L  L  P  L  D  R

63901 CTGCCGCTGACCGCCAACGGCAAGGTCGACCGGGCCGCACTCCAACGCCTCGTCCCCGGC 63960
      L  P  L  T  A  N  G  K  V  D  R  A  A  L  Q  R  L  V  P  G

63961 CGCGCACCGGCCCCGGCGGAACCCGCCACCGCCCCACCTGCCCGTTCCCGCGCCGTCCCC 64020
      R  A  P  A  P  A  E  P  A  T  A  P  P  A  R  S  R  A  V  P

64021 GTGCCCGGCTGGCTCGCCGACCTGTGGTGCGAACTCCTCGACGTGCCGGAGGCCGACCCC 64080
      V  P  G  W  L  A  D  L  W  C  E  L  L  D  V  P  E  A  D  P

64081 GACGCGAACTTCTTCGCCCTCGGCGGCACCTCCCGGGTCGCGATCACCCTGGTCACCCGG 64140
      D  A  N  F  F  A  L  G  G  T  S  R  V  A  I  T  L  V  T  R

64141 ATCGAGGCCCGACTCGCCGTCCGGGTGCCCCTCGCCCGCCTCTTCGACGCCCGCACCCTG 64200
      I  E  A  R  L  A  V  R  V  P  L  A  R  L  F  D  A  R  T  L

64201 GGCGGCCTCGCCGAGACGATCGCCGAACTGTCGGCCGCCGCCGAGGAGGAGCCGGCACCC 64260
      G  G  L  A  E  T  I  A  E  L  S  A  A  A  E  E  E  P  A  P

64261 GCCGAGCCCGTGTACGCCCCCGACCCCGCCACCCGCCACGAGCCGTTCCCGCTCACCGAC 64320
      A  E  P  V  Y  A  P  D  P  A  T  R  H  E  P  F  P  L  T  D

64321 ATCCAGCGCGCCTACTGGCTCGGCCGGCACCGCTCCCTCTCCCTTGGCGGCGTCGCCACG 64380
      I  Q  R  A  Y  W  L  G  R  H  R  S  L  S  L  G  G  V  A  T

64381 CACACCTACCTCGAACTCGACGTCGAGGACCTCGACCCCGGCCGGCTCCAGACGGCCCTC 64440
      H  T  Y  L  E  L  D  V  E  D  L  D  P  G  R  L  Q  T  A  L

64441 CGCCGGCTGATCGACCGCCACGACGCCCTCCGGCTCGTGGTCCTCCCCGACGGCCGGCAA 64500
      R  R  L  I  D  R  H  D  A  L  R  L  V  V  L  P  D  G  R  Q
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64501 CAGATCCTCGGCGACGTACCGCCGTACCTCCTCGCCCACACCGACCTGCGGGGCAGGGCG 64560
      Q  I  L  G  D  V  P  P  Y  L  L  A  H  T  D  L  R  G  R  A

64561 GACGCCGAGGCCGAACTGGCCCGCGTCCGCGAGCACATGTCGCACGAGGTGCGCGACGCC 64620
      D  A  E  A  E  L  A  R  V  R  E  H  M  S  H  E  V  R  D  A

64621 TCCCGCTGGCCGCTGTTCGACGTACGGACCCACCGCCTGGACGACGTCCGCACCCGGCTG 64680
      S  R  W  P  L  F  D  V  R  T  H  R  L  D  D  V  R  T  R  L

64681 CACCTGAGCTTGGACCTGCTCATCGCCGACGCCCACAGCGTCCACGTACTCACCGGCGAC 64740
      H  L  S  L  D  L  L  I  A  D  A  H  S  V  H  V  L  T  G  D

64741 CTGCTCACCTTCTACGCCGACCCCGACGCGGCCCTGCCGCCCCTCGGCTGCTCCTTCCGC 64800
      L  L  T  F  Y  A  D  P  D  A  A  L  P  P  L  G  C  S  F  R

64801 GACTACGTCCTGGCCGTCCGCGCCCACGCCGAGGGCGAGCCGCGCCGCCGCGCCCTCGAC 64860
      D  Y  V  L  A  V  R  A  H  A  E  G  E  P  R  R  R  A  L  D

64861 CACTGGCGGGCCCGGCTGGCCGACCTGCCGGGCCCGCCCGGCCTGCCGCTGCGGTGCCGG 64920
      H  W  R  A  R  L  A  D  L  P  G  P  P  G  L  P  L  R  C  R

64921 CCCGAGGAGCTGACCGCGCCGCGGTTCGCCCGCCTCACCACCGGACTCGGCCCCGACGCC 64980
      P  E  E  L  T  A  P  R  F  A  R  L  T  T  G  L  G  P  D  A

64981 TGGGCACGGCTGCGGCGCGCCGCGGCGGCCGCCGAACTCACCCCGGCCGCACTGATCTGC 65040
      W  A  R  L  R  R  A  A  A  A  A  E  L  T  P  A  A  L  I  C

65041 GCCGCCTTCTGCGACGTCCTCGCCCAGTGGAGCGACACCCCCCGCTTCACCCTCAACCTC 65100
      A  A  F  C  D  V  L  A  Q  W  S  D  T  P  R  F  T  L  N  L

65101 ACCACCTTCCACCGCCCCGCCCTGCTCCCCGGCGTGGACGACCTCGTCGGCGACTTCACC 65160
      T  T  F  H  R  P  A  L  L  P  G  V  D  D  L  V  G  D  F  T

65161 ACCACGACCCTGCTCGGGGTCGACGGCGAGGGGGACACCTTCCGGGACCGGGCCCGCCGA 65220
      T  T  T  L  L  G  V  D  G  E  G  D  T  F  R  D  R  A  R  R

65221 CTCCAGGACCGCATCTGGGAGGACCTCGAACACCGCGTCGTCAGCGGCGTCGAGGTCCTG 65280
      L  Q  D  R  I  W  E  D  L  E  H  R  V  V  S  G  V  E  V  L

65281 CGGATGCTGCGCCGCGAGCGGGGCACCCACGACGCCGTCCGGATGCCGGTCGTCTTCACC 65340
      R  M  L  R  R  E  R  G  T  H  D  A  V  R  M  P  V  V  F  T

65341 AGCACCCTGCGGGCCGCCGGCCCCGCCCCCCGGACGGCCCCGCCCGCCTGGCGGGTACGG 65400
      S  T  L  R  A  A  G  P  A  P  R  T  A  P  P  A  W  R  V  R

65401 CCCGGCTACGCGATCAGCCAGACCCCGCAGGTCCTGCTCGACCATCAGGTGAGCGAGAGC 65460
      P  G  Y  A  I  S  Q  T  P  Q  V  L  L  D  H  Q  V  S  E  S

65461 GACGGCCGACTGGTCTGCACCTGGGACTACGTCGCGGACGCCTACCCGCCCGGGCTGATC 65520
      D  G  R  L  V  C  T  W  D  Y  V  A  D  A  Y  P  P  G  L  I

65521 GAGGCCATGTTCGGGGCCTTCGAGGCGCTCCTCGCCTCGCTCGCCGGTCACGACGACGAC 65580
      E  A  M  F  G  A  F  E  A  L  L  A  S  L  A  G  H  D  D  D

65581 GCCGGCCACGACGACGACGCCGGCCACGACGACGGCCCCGGCCACGACGACGGCCCCGGC 65640
      A  G  H  D  D  D  A  G  H  D  D  G  P  G  H  D  D  G  P  G

65641 CACGACGACGGCCCCGGCCACGACGACGGCCCCGGCCACGACGACGGCCCCGGCCGCGAC 65700
      H  D  D  G  P  G  H  D  D  G  P  G  H  D  D  G  P  G  R  D

65701 GACAGTGCCGATCACGGCCACAGTGCCACGCACGACGACAGCGCCGCCCGAAACGACAGA 65760
      D  S  A  D  H  G  H  S  A  T  H  D  D  S  A  A  R  N  D  R

65761 GAGGGAGGTGGACCGGAGTGACGAGCGCCCGGCCCACGCCGACACTGCTCCCCGCCGACC 65820
      E  G  G  G  P  E  *
                        M  T  S  A  R  P  T  P  T  L  L  P  A  D  Q  (orf16)

65821 AGCGGGAGCTGCTGCGGATGATGAACGACCGCACCGCACCCGTGCCCGCGCACACCCTCA 65880
        R  E  L  L  R  M  M  N  D  R  T  A  P  V  P  A  H  T  L  T

65881 CCGCCCAACTGGCCGACGCCGCGCGCACGCACGACCGGGCTCTGGCACTGGTGGCACCGG 65940
        A  Q  L  A  D  A  A  R  T  H  D  R  A  L  A  L  V  A  P  G
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65941 GTCTGACACTGAGCCACGCCGAACTGGACGCCCGGGCGGCCGCGGTGGCCGCCCGGCTCA 66000
      L  T  L  S  H  A  E  L  D  A  R  A  A  A  V  A  A  R  L  T

66001 CCGCCGCGGGCGTCATCCCCGGGGACCGGGTCGCCCTCGCCGTCGAGTACGGCTGGGAGC 66060
      A  A  G  V  I  P  G  D  R  V  A  L  A  V  E  Y  G  W  E  Q

66061 AGGTCGTGGGCGCCCTGGCCGCGCTCCGCGCCGGAGCCGTCTGCCTGCCCGTCGCCCCCG 66120
      V  V  G  A  L  A  A  L  R  A  G  A  V  C  L  P  V  A  P  G

66121 GGCTGCCCCGGCCCGCCCGCTGGCAGCACGCCACCCGGGCCGGGGCGACGGCCGTCCTCA 66180
      L  P  R  P  A  R  W  Q  H  A  T  R  A  G  A  T  A  V  L  T

66181 CCCAGTCCTGGCTCACCCAGCGCATCGACTGGCCGCAGGAACTGCCCGTCCTCTCCGTGG 66240
      Q  S  W  L  T  Q  R  I  D  W  P  Q  E  L  P  V  L  S  V  D

66241 ACGAACCCGGGCCGCCGGTACCACCCACCACCGCCCCGGCCGACGGACGGTCCGCGACCG 66300
      E  P  G  P  P  V  P  P  T  T  A  P  A  D  G  R  S  A  T  D

66301 ACGCCGCCTACCGGCTGGACGCCCCCGTCAGCCACCGCGCGATCACCACCGCCGCCCTGG 66360
      A  A  Y  R  L  D  A  P  V  S  H  R  A  I  T  T  A  A  L  E

66361 AGATCGACCGCGCCTTCCGCGTCGGACCCGGCGACCGGCTCCTGGCCCTGGCCCCCGCCG 66420
      I  D  R  A  F  R  V  G  P  G  D  R  L  L  A  L  A  P  A  D

66421 ACTCGCCGCTCGCTCTCTACGAACTGTTCGGGCCCCTCCTGGCCGGTGCGGCCCTCGTCC 66480
      S  P  L  A  L  Y  E  L  F  G  P  L  L  A  G  A  A  L  V  L

66481 TCACCCGGGACATCGACCTGCGCGATCCCGGAGCCCTGCACGAGGCGCTGCGCACCCACG 66540
      T  R  D  I  D  L  R  D  P  G  A  L  H  E  A  L  R  T  H  G

66541 GCGTCACCCTCTGGCACTCGCCGCCCGCCCTCCTCGGCCTCCTCCTCGACCACCTCGCCG 66600
      V  T  L  W  H  S  P  P  A  L  L  G  L  L  L  D  H  L  A  D

66601 ACCGGGGCGGCAAACTGCCCGAGTCGCTCCGGCTGGTGCTGCTCGGCGGCGAACGCCTCG 66660
      R  G  G  K  L  P  E  S  L  R  L  V  L  L  G  G  E  R  L  D

66661 ACCCCGCCCTCGTCCGCCGCGTCCGCGAGAGCGCCCCGCACCAGCCGGCCGTCGCCCACC 66720
      P  A  L  V  R  R  V  R  E  S  A  P  H  Q  P  A  V  A  H  L

66721 TCTCCTCGGCCACCCCGTCCGGCCCCTGGACCACCTGCCTGGAGACCGGCGACCTCGCCC 66780
      S  S  A  T  P  S  G  P  W  T  T  C  L  E  T  G  D  L  A  P

66781 CGGAATGGCGCTCGGTCCCCGTCGGCGCGCCCCTGCCCAACCAGCGGGCGCACATCCTGT 66840
      E  W  R  S  V  P  V  G  A  P  L  P  N  Q  R  A  H  I  L  S

66841 CCGAGACCCTGCGGCCCTGCCCGGTCTGGGTCACCGGCCGCCTCCACTACGGCGGCGTCG 66900
      E  T  L  R  P  C  P  V  W  V  T  G  R  L  H  Y  G  G  V  A

66901 CCGCCGAGCCCCCCACCGGAGAGGAGCACGCACCCGCGACCGTCCCGCACCCGGAGACCG 66960
      A  E  P  P  T  G  E  E  H  A  P  A  T  V  P  H  P  E  T  G

66961 GCGAACCGCTGCTGCGCACCGGGCTGTTCGCCCGCCTGCTGCCCGAGGGCCTGATCGACG 67020
      E  P  L  L  R  T  G  L  F  A  R  L  L  P  E  G  L  I  D  V

67021 TCGTCGGCGACGAGACCGCCCGGATCAGCGTCCGCGACCGGCCCCTGAACCTCCAGGACA 67080
      V  G  D  E  T  A  R  I  S  V  R  D  R  P  L  N  L  Q  D  T

67081 CCGAGACCGCCCTCGCCGCCCACGAGGACGTGCACTCCGCCGTGGTCGTCCCCGTCGGGC 67140
      E  T  A  L  A  A  H  E  D  V  H  S  A  V  V  V  P  V  G  R

67141 GGGGAGACGAGTCGCTCGCGCGGGTACGGCTCCACCCCGGCGCCACGGCCGGCCCCGACG 67200
      G  D  E  S  L  A  R  V  R  L  H  P  G  A  T  A  G  P  D  E

67201 AACTCCTCGCCCATCTGCGCCGCAAGGTCTCCCCTTACCTGCTGCCCGGCCACATCGAGG 67260
      L  L  A  H  L  R  R  K  V  S  P  Y  L  L  P  G  H  I  E  V

67261 TGGGCGGTCCGCTGCCGCTCACCCGGGACGGGCGCGTGGACCGCGCGCGCGTCACCGCCG 67320
      G  G  P  L  P  L  T  R  D  G  R  V  D  R  A  R  V  T  A  E

67321 AGGCCCCCGCCCCCGCTGCCGTGCCCGCCGCCGCGCCGGCGGCGTCGGCACCCGCGCGGG 67380
      A  P  A  P  A  A  V  P  A  A  A  P  A  A  S  A  P  A  R  D

67381 ACGAGGCCGAACTCCTCGCCCAAGTGGCCCGGGTGACCTGCCGGGTGCTGGGAATCGGCG 67440
      E  A  E  L  L  A  Q  V  A  R  V  T  C  R  V  L  G  I  G  A
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67441  CCGTCGAACCCGATATGAACCTGCTCGACGCCGGTGCCACCTCCGTCGAACTCGTCCGCC  67500
         V  E  P  D  M  N  L  L  D  A  G  A  T  S  V  E  L  V  R  L

67501  TGGCGACCGCTCTGGAGGAGGAACTCGGCCTCGACACCGACATCGAGGAACTGCTGGCCT  67560
         A  T  A  L  E  E  E  L  G  L  D  T  D  I  E  E  L  L  A  F

67561  TCCCGTCGGTCGCCGTGATCGTCGGCCGCCACCTCGGCCGCCGGACGGCACCACCGGCCC  67620
         P  S  V  A  V  I  V  G  R  H  L  G  R  R  T  A  P  P  A  R

67621  GGGACCCCCTGCCGCCCGCGTCCGTAGCGTTCGCACCCGGGTCCGTACTGCCCGCGCCGC  67680
         D  P  L  P  P  A  S  V  A  F  A  P  G  S  V  L  P  A  P  P

67681  CCGCGCCCGGACCCGTGCCGCCCGCGTCCGTGCCGCCCGCACCCGCGTCCGTACCGCCCG  67740
         A  P  G  P  V  P  P  A  S  V  P  P  A  P  A  S  V  P  P  A

67741  CGTCCGAGTCCTCACCGCTCGCGCCGCCCGCACCCGGGCCCGTGCCACCCACGCCCGTCC  67800
         S  E  S  S  P  L  A  P  P  A  P  G  P  V  P  P  T  P  V  P

67801  CGCCCGCCTCCGTCCCGCCCGCGTCCGGGGCCGCGCCGCACGTACCGCCCGCGCCGCCCG  67860
         P  A  S  V  P  P  A  S  G  A  A  P  H  V  P  P  A  P  P  A

67861  CACCCATCCCCGCGCCCTCCGTGCCccccgcgccccgccccccaaccgcccctgctcaccg  67920
         P  I  P  A  P  S  V  P  P  A  P  R  P  Q  P  P  L  L  T  G

67921  gcatcggcgcccgccaggcgTTCAAGGACGCCCACCACGGCATCCGGCACGAGTTCGACG  67980
         I  G  A  R  Q  A  F  K  D  A  H  H  G  I  R  H  E  F  D  A

67981  CCACCGACGGCGTCGCCCTCAGCGGCCCGGACGACCACCACCTCACCGCCCGTCGCAGCC  68040
         T  D  G  V  A  L  S  G  P  D  D  H  H  L  T  A  R  R  S  H

68041  ACCACCGCTTCGACCCCGGCCCCGTGACGCTGCCGGACCTGGCCGCCCTCCTCGGGGCCC  68100
         H  R  F  D  P  G  P  V  T  L  P  D  L  A  A  L  L  G  A  L

68101  TCCGCCGGGTCCGCGGCCCGGGAGGCGAACCCAAATACGCCTATCCGTCGGCCGGTTCCT  68160
         R  R  V  R  G  P  G  G  E  P  K  Y  A  Y  P  S  A  G  S  S

68161  CCTACCCCGTCCAGACCTACCTGCTCGTCCACCCGGGGAAGGTGACCGGACTGCCCGGCG  68220
         Y  P  V  Q  T  Y  L  L  V  H  P  G  K  V  T  G  L  P  G  G

68221  GCAGCCACTACGTCCACCCCGCGCGCAACCGCCTGGTGAGCATCGACCCCACCGCGACCC  68280
         S  H  Y  V  H  P  A  R  N  R  L  V  S  I  D  P  T  A  T  L

68281  TGCCCGCCGACGCGCACGCCGAGATCAACCGCGCCGCCTACGGGGAGGCGGCCTTCTCCC  68340
         P  A  D  A  H  A  E  I  N  R  A  A  Y  G  E  A  A  F  S  L

68341  TCTACCTCATCGCCGCGATCGACGCGATCACACCGCTCTACGGCGATCTCTCCTGGGACT  68400
         Y  L  I  A  A  I  D  A  I  T  P  L  Y  G  D  L  S  W  D  F

68401  TCACCGTCTTCGAGGCCGGTGCCATGACCCAGTTGCTGATGCGGACCGCCGTCGGCACCG  68460
         T  V  F  E  A  G  A  M  T  Q  L  L  M  R  T  A  V  G  T  G

68461  GCATCGGCCTGTGCCCCGTCGGCACGATGGACCCCGCGCCGCTGCGCCGCGCGTTCGCCC  68520
         I  G  L  C  P  V  G  T  M  D  P  A  P  L  R  R  A  F  A  L

68521  TCACCGACCGGCACCGCTTCGTCCACGCCCTCCTCGGCGGGCGGCCCCGCACGGAGGCCC  68580
         T  D  R  H  R  F  V  H  A  L  L  G  G  R  P  R  T  E  A  P

68581  CGTGAACCGGCACGGCCCCCTGGCGGGCCGGCGGCAGAGCGTCGACACCCGCAGCGCCGC  68640
         M  N  R  H  G  P  L  A  G  R  R  Q  S  V  D  T  R  S  A  A   (orf15)
         *

68641  GTGGGTGGCGCCGACGGGCACCCCGGGGCTGCCGCTGGAGGTGGCCGCCACCCGGGACGG  68700
         W  V  A  P  T  G  T  P  G  L  P  L  E  V  A  A  T  R  D  G

68701  CGTCGACCCGGCCGAATGGGCCCGCACCCACCTCGACACCGTCACCGGCTGGCTGCACCG  68760
         V  D  P  A  E  W  A  R  T  H  L  D  T  V  T  G  W  L  H  R

68761  TCACGGAGCCGTCCTGTTCCGCGGCTTCGGCGTCGGCCTCGACGGCTTCGGCGACGTCGT  68820
         H  G  A  V  L  F  R  G  F  G  V  G  L  D  G  F  G  D  V  V

68821  CCACGCCCTGGCCGGATCCCCCGAGGCGTACGTCGAACGGTCGTCGCCGCGCACCGCCCT  68880
         H  A  L  A  G  S  P  E  A  Y  V  E  R  S  S  P  R  T  A  L
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68881  CGGGCATCACCTCTACACCGCCACCGACCACCCCGCCGACCAGCCCATCCCCCCGCACAA  68940
         G  H  H  L  Y  T  A  T  D  H  P  A  D  Q  P  I  P  P  H  N

68941  CGAGAACTCCTACCAACTCCGCTTCCCCGGACGGCTGGTCTTCGGCTGCCTCACCCCGGC  69000
         E  N  S  Y  Q  L  R  F  P  G  R  L  V  F  G  C  L  T  P  A

69001  CCGGACCGGCGGCGCGACCCCGCTCGCCGACACCCGGCGCGTCCTGGGCCGCCTCGACCC  69060
         R  T  G  G  A  T  P  L  A  D  T  R  R  V  L  G  R  L  D  P

69061  CGCCCTCGTCGCCGCCTTCGCCCGCCGCGGGGTGCTCTACCAGCGCAACTACGGCGACGG  69120
         A  L  V  A  A  F  A  R  R  G  V  L  Y  Q  R  N  Y  G  D  G

69121  GATCGGCATGTCCTGGCAGGACGCCTTCCAGACCCGCGACAAGGCGGCCGTCACCGCCTA  69180
         I  G  M  S  W  Q  D  A  F  Q  T  R  D  K  A  A  V  T  A  Y

69181  CTGCGCCGCCCGCCGCGTCGACGTCGAATGGAAACCCGACGGCGGGCTGCGGACCACCCA  69240
         C  A  A  R  R  V  D  V  E  W  K  P  D  G  G  L  R  T  T  Q

69241  GGTCCGCCCCGCCCTCGCCGTCCACCCGGCGACGGGGGAGCGGGTGTGGTTCAACCACGC  69300
         V  R  P  A  L  A  V  H  P  A  T  G  E  R  V  W  F  N  H  A

69301  CGCGTTCTTCCACGTCTCCGCCCGGCCGCCCGCGCTGCGGGACGCCCTGCTGGCCCAGTT  69360
         A  F  F  H  V  S  A  R  P  P  A  L  R  D  A  L  L  A  Q  F

69361  CGACGAACGCGACCTGCCGAGCCACTCCTGCTACGGCGACGGCCGGCCCATCGAACCCGC  69420
         D  E  R  D  L  P  S  H  S  C  Y  G  D  G  R  P  I  E  P  A

69421  CGTCATGGAGGAACTGCACCACGCCTACGCCGCCGAACTGGTGGCGCCCGCCTGGCGGGC  69480
         V  M  E  E  L  H  H  A  Y  A  A  E  L  V  A  P  A  W  R  A

69481  CGGCGACGTCCTCCTCGTCGACAACCTCCTCACCGCGCACGGCAGGGAACCCTTCACCGG  69540
         G  D  V  L  L  V  D  N  L  L  T  A  H  G  R  E  P  F  T  G

69541  CGAACGCCGCGTCGTCGTCGGCATGGCACAGCCGCTGGACTGGGACGAGGTGAGCGCGTG  69600
         E  R  R  V  V  V  G  M  A  Q  P  L  D  W  D  E  V  S  A  *
                                                                M     (orf14)

69601  ACCGCCCCCGGCACACCGCTGCCCGCGACCTTCGTCCAGCGCGGCCTGTGGCCGTCCACT  69660
         T  A  P  G  T  P  L  P  A  T  F  V  Q  R  G  L  W  P  S  T

69661  CGCCACGCCCGCCCGGCGGAGGTCACCCACGTCCGCGCCCTGCGCCTGACCGGGGACACC  69720
         R  H  A  R  P  A  E  V  T  H  V  R  A  L  R  L  T  G  D  T

69721  GACACGGCGCGGCTCACCGAGGCCGTCCGGCGGGTCACCGCCGCCCTCCCCGCCCTCACC  69780
         D  T  A  R  L  T  E  A  V  R  R  V  T  A  A  L  P  A  L  T

69781  GCCGAACTCTCCGGCGACGAGGAACCCCGCCTGACCCTCCGGCCGGACGCCCCCGAGGTC  69840
         A  E  L  S  G  D  E  E  P  R  L  T  L  R  P  D  A  P  E  V

69841  ACCCCGGTCGACCTGCGCGGAGCCCCGTCCGCCGGACGCGACGCCGTCTGCGTGGCGCTG  69900
         T  P  V  D  L  R  G  A  P  S  A  G  R  D  A  V  C  V  A  L

69901  CTGCGCGCCGACCGGGACCACCCTCGCGCCGGACGCCACCGGGCCCGCTTCCACCTGGTG  69960
         L  R  A  D  R  D  H  P  R  A  G  R  H  R  A  R  F  H  L  V

69961  CGGCTCCACGACGACGAGACGGTGCTCGCGCTCACGGCCCACACCCTCCTCCTCGACACA  70020
         R  L  H  D  D  E  T  V  L  A  L  T  A  H  T  L  L  L  D  T

70021  CCGTCTCTCTACGCCGTGCTCGGCGCGGTCTGCCAGGCGTACGCCGGCCGCTTCCGCCCC  70080
         P  S  L  Y  A  V  L  G  A  V  C  Q  A  Y  A  G  R  F  R  P

70081  GAGCACTACCGCGACGCCACCACCCTGCCCGACGCGCCCCACGCCCCCCTCTCCGGTCGG  70140
         E  H  Y  R  D  A  T  T  L  P  D  A  P  H  A  P  L  S  G  R

70141  GCCCGGGCCTCCCGCCGGCGCTGGTGGCACCGGCGCCTGGCCGCCCTGCCCGGCCCGGCC  70200
         A  R  A  S  R  R  R  W  W  H  R  R  L  A  A  L  P  G  P  A

70201  CCGGCCCCCGCCGGCCCGCCCCGCGACCGGGTGACCGAAACCCACCGGCTGCGCATCCCC  70260
         P  A  P  A  G  P  P  R  D  R  V  T  E  T  H  R  L  R  I  P

70261  GCAGCGCGCTGGAAAGCCCTGACCGCCCTGACCGCCCTGGGCGGCCCCCTCGGCGGCAAC  70320
         A  A  R  W  K  A  L  T  A  L  T  A  L  G  G  P  L  G  G  N

70321  GGCTCGCTCGCCGTCATGGCCCTGGCCGCCTGGTGCCTGCGCGCCCCGGACCACCGGGGA  70380
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          G  S  L  A  V  M  A  L  A  A  W  C  L  R  A  P  D  H  R  G

70381 CCGGCCCGCTTCACCACCGTCGTCGACCTGCGCGACCACCTCGGACTCGGGCCCGCCGTC 70440
      P  A  R  F  T  T  V  V  D  L  R  D  H  L  G  L  G  P  A  V

70441 GGCCCGTTCACCGACCGCCTCGTCTTCGGCGCCGACCTCGGCGAAGCGCCGCGCCCCTCC 70500
      G  P  F  T  D  R  L  V  F  G  A  D  L  G  E  A  P  R  P  S

70501 TTCCGGGACGTCACGCTGCGCGCCCAGTCCGGGTTCCTGGACGCCGTCGTGCACTACCTC 70560
      F  R  D  V  T  L  R  A  Q  S  G  F  L  D  A  V  V  H  Y  L

70561 CCCTACGGCGACGTCGTGGAACTCgGCAGGGAACTGGGCCGCGTCACCGCGCCCCGCACC 70620
      P  Y  G  D  V  V  E  L  G  R  E  L  G  R  V  T  A  P  R  T

70621 GCCGCGCACTGGGACGTGGCGCTGAACTTCTGCCGCAACCCGCCCACCAGCGCCGCCACC 70680
      A  A  H  W  D  V  A  L  N  F  C  R  N  P  P  T  S  A  A  T

70681 CGCGGCGAACGCACCCTCGCCGAACGCGGCCTGTCCATCGAGCTGTTCCGCGAGGCCGAC 70740
      R  G  E  R  T  L  A  E  R  G  L  S  I  E  L  F  R  E  A  D

70741 CTGCTCGGCGCGGCCGGCACCGGTCCCGCGCACCGGTGGGACGGCACGGTGCTCGCCCTC 70800
      L  L  G  A  A  G  T  G  P  A  H  R  W  D  G  T  V  L  A  L

70801 TCCCTAGGCGAACTCGGCGACGACACCGTGCTGGTCCTCGACGCCGACCGCGACCACCCG 70860
      S  L  G  E  L  G  D  D  T  V  L  V  L  D  A  D  R  D  H  P

70861 CACCACGGAACCGCCGACCGGCTGCTCCACCGGATGGACGAAGCGCTCCTGGCGGCCGTC 70920
      H  H  G  T  A  D  R  L  L  H  R  M  D  E  A  L  L  A  A  V

70921 GCCGACCCGGACGCCCCCCTGCCCCCCTTGCCCGCCCCCGCGCACACCACGAGGAGCCAC 70980
      A  D  P  D  A  P  L  P  P  L  P  A  P  A  H  T  T  R  S  H

70981 CGATGACCACGACCCCGCGGACCGCCGCCGAGCCCACCTACCACGTGGTGGTCAACGACG 71040 (orf13)
        M  T  T  T  P  R  T  A  A  E  P  T  Y  H  V  V  V  N  D  E
      R  *

71041 AGGAGCAGTACTCGATCTGGCTCGCCGAACAGGAGATCCCGGCCGGCTGGCGGGCCACCG 71100
        E  Q  Y  S  I  W  L  A  E  Q  E  I  P  A  G  W  R  A  T  G

71101 GAACCTCCGGCACCCAGGAGGAGTGCCTGCGCCACATCGACGAGGTGTGGACCGACATGC 71160
        T  S  G  T  Q  E  E  C  L  R  H  I  D  E  V  W  T  D  M  R

71161 GCCCCCGCAGCCTGCGCGAGGCCATGGCCGCGGCGGAGCACGCGGAGCCCGCTCCCGCCC 71220
        P  R  S  L  R  E  A  M  A  A  A  E  H  A  E  P  A  P  A  P

71221 CGGCCCCGGCCGAGGAGGAGCCGAGCCTCGTCGACCGGCTCTGCGCGGGCGACCAGCCGG 71280
        A  P  A  E  E  E  P  S  L  V  D  R  L  C  A  G  D  Q  P  V

71281 TGGAGTCGGTCCTCCGCCCGGAGCGCACGGCCGCCGCCCTGCGGGAGGCCGTCGACCGCG 71340
        E  S  V  L  R  P  E  R  T  A  A  A  L  R  E  A  V  D  R  G

71341 GCTACGTCTTCGTCCGCTTCGCCGCCACCCGCGGCGGCACCGAACTCGGCGTCGCCGTCG 71400
        Y  V  F  V  R  F  A  A  T  R  G  G  T  E  L  G  V  A  V  D

71401 ACCCCGCGGCGACCACCATGGACGGCACCGAGCTGCGCCTGACCGGCACCCTCACCCTCG 71460
        P  A  A  T  T  M  D  G  T  E  L  R  L  T  G  T  L  T  L  D

71461 ACTTCGAACCGGTCCGCTGCCACGCCCGCGTCGACGTGACCACCTTCACGGGCGAGGGCC 71520
        F  E  P  V  R  C  H  A  R  V  D  V  T  T  F  T  G  E  G  R

71521 GCCTGGAGCGCGTGTCCGGCACCTGACCCCCGCCGGCCACCCGGCCGTGAGGCGCGGCTC 71580
        L  E  R  V  S  G  T  *

71581 GGGACCGGGCCGCCGACCCACCGAAGGGAGGGACCCCATGACCACCCCCATGACCACCCC 71640 (orf12)
                                    M  T  T  P  M  T  T  P

71641 CACGACCACCCGCACCACCACCCGCACCGCCGTCTTCGCCCACCTCCGCGCCCCCGGCCT 71700
        T  T  T  R  T  T  T  R  T  A  V  F  A  H  L  R  A  P  G  L

71701 CGGCGACCTCCTCCAGCGCAACATCGGCCTCGCCCTCGTCCGCCGCGCCCGCCCGGCGAC 71760
        G  D  L  L  Q  R  N  I  G  L  A  L  V  R  R  A  R  P  A  T

71761 GGCGGTCACCCTGGTCGTCGGCGAGGACCTGGCGGCCCGCTTCGGTCCGGCACTCACCCG 71820
        A  V  T  L  V  V  G  E  D  L  A  A  R  F  G  P  A  L  T  R
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71821 CCACACGTACGCCACCGACGTGCTGCCCTGCCCCCAGCGGGGCGAGGCCGACCCCCGGTG 71880
      H  T  Y  A  T  D  V  L  P  C  P  Q  R  G  E  A  D  P  R  W

71881 GCCCGCCTTCCTGCGCACCCTGGCCGACCGCCGCTTCGCCCTCGCCGTCGTCGACCCGGA 71940
      P  A  F  L  R  T  L  A  D  R  R  F  A  L  A  V  V  D  P  D

71941 CAGCCAGGGCCTGCACGCCGGCCACGCCCGGGCCGCCGGCGTGCCCGAGCGGATCGGCCT 72000
      S  Q  G  L  H  A  G  H  A  R  A  A  G  V  P  E  R  I  G  L

72001 GCCGCAGGACCGGCCCGGAGACGAACACATCACCCATCCCATCCGCCTCCCACGTCCCCT 72060
      P  Q  D  R  P  G  D  E  H  I  T  H  P  I  R  L  P  R  P  L

72061 GTGGGGGACCCCGGACCTGTACGAGTACGCCACTGCCCTCGCCGCCGCGCTGGGCCTGCC 72120
      W  G  T  P  D  L  Y  E  Y  A  T  A  L  A  A  A  L  G  L  P

72121 CGCACCGCCGCGCCCCGGCGACGTCCTGCCGGAGCTGCCCCGCACCCGCGGCGTCCGCCC 72180
      A  P  P  R  P  G  D  V  L  P  E  L  P  R  T  R  G  V  R  P

72181 GCCGACGGCCGGTCTGCCCCGTCCGCTCGTCGCCGTCCACCCCGGCGGGGCACCGCACTG 72240
      P  T  A  G  L  P  R  P  L  V  A  V  H  P  G  G  A  P  H  W

72241 GAACAGGAGATGGCCGCTCGAGCACTACGCCCGGCTCTGCGCCCGCCTCGCGGCCGAACT 72300
      N  R  R  W  P  L  E  H  Y  A  R  L  C  A  R  L  A  A  E  L

72301 CTCGGCCTCCCTCTGCCTGCTGGGCGACGAAGCCGAACGCCCCGAGCTGGAACTGCTCCG 72360
      S  A  S  L  C  L  L  G  D  E  A  E  R  P  E  L  E  L  L  R

72361 GCACGCCGTCCTGACGCGGTCCCCGCGAGCCGTCGTCCACCTCGAGGCGGGCGCGGACCT 72420
      H  A  V  L  T  R  S  P  R  A  V  V  H  L  E  A  G  A  D  L

72421 CGACCGGACCGCGAACGTCCTCGCCGACGCCGACCTGCTCGTCGGCAACGACTCCTCGCT 72480
      D  R  T  A  N  V  L  A  D  A  D  L  L  V  G  N  D  S  S  L

72481 CGCCCACGTCGCCGCCGCCGTCCGCACCCCGTCCGTCGTCCTCTACGGCCCGACCGGCAC 72540
      A  H  V  A  A  A  V  R  T  P  S  V  V  L  Y  G  P  T  G  T

72541 CGAGTACCTGTGGACCAGGATCTACCCGTACCACCGCGGGGTCTCCCTGCGGTGGCCGTG 72600
      E  Y  L  W  T  R  I  Y  P  Y  H  R  G  V  S  L  R  W  P  C

72601 CCAGCGGCTGCGGCACGCCGCAGGCGAACTCGCCGGCCGGCGGTGCGCGCACGGCTGCGT 72660
      Q  R  L  R  H  A  A  G  E  L  A  G  R  R  C  A  H  G  C  V

72661 CCTGCCCTACCAGGGCCCGGCCGGCCCGTATCCGCGCTGTCTGGCCGACCTGCCGGTGGA 72720
      L  P  Y  Q  G  P  A  G  P  Y  P  R  C  L  A  D  L  P  V  D

72721 CAGGGTCTGGCCGGCGGTGACCGCCCGATGGGCGAGCCCCCACCCCGTGACGATCAGGAG 72780
      R  V  W  P  A  V  T  A  R  W  A  S  P  H  P  V  T  I  R  S

72781 TACCCCATGAGCGCCGACCCGTCCCGGGTGCGGACGATCCTCTCCGTCAACTTCAACCAC 72840
      T  P  *
            M  S  A  D  P  S  R  V  R  T  I  L  S  V  N  F  N  H  (orf11)

72841 GACGGCTCCGGCGTGCTGTTGCGGGAGGGCAGGATCGCCGGCTACGTCACCACCGAGCGC 72900
      D  G  S  G  V  L  L  R  E  G  R  I  A  G  Y  V  T  T  E  R

72901 CGCTCCCGCCTCAAGAAGCACCCGGGCCTGCGCGAGGAGGACCTCGACGAACTGCTGGAC 72960
      R  S  R  L  K  K  H  P  G  L  R  E  E  D  L  D  E  L  L  D

72961 CAGGCCGGGGCCGACCTCTCCGACATCGACCACGTCATGCTCTGCAACCTGCACACCATG 73020
      Q  A  G  A  D  L  S  D  I  D  H  V  M  L  C  N  L  H  T  M

73021 GACACACCCGACATACCCCGGCTGCACGGCTCCGACCTCAAGGAGACCTGGCTCGCGTTC 73080
      D  T  P  D  I  P  R  L  H  G  S  D  L  K  E  T  W  L  A  F

73081 TGGGTCAACCAGCGCAACGACGAGGTGAGCCTGCGCGGCCGCCGCATCCCCTGCACCGTC 73140
      W  V  N  Q  R  N  D  E  V  S  L  R  G  R  R  I  P  C  T  V

73141 AACCCGGACCACCACCTCATCCACGCCGCCACCGCCTACTACACCTCCGGCTACGACTCG 73200
      N  P  D  H  H  L  I  H  A  A  T  A  Y  Y  T  S  G  Y  D  S

73201 GCGATGGCCGTGGCCATCGACCCCACCGGCTGCCGCGCCTTCGCCGGCAAGGGCAGCCGC 73260
      A  M  A  V  A  I  D  P  T  G  C  R  A  F  A  G  K  G  S  R
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73261  CTCTACCCCCTGCGCCGCGACCTCGACGCCTGGTTCAACGCCAACATCGGCTACTGCTAC  73320
       L  Y  P  L  R  R  D  L  D  A  W  F  N  A  N  I  G  Y  C  Y

73321  GTCGCCGACCTGATGTTCGGCTCCAGCATCGTCGGCGCCGGCAAGGTCATGGGCCTCGCC  73380
       V  A  D  L  M  F  G  S  S  I  V  G  A  G  K  V  M  G  L  A

73381  CCCTACGGCAGACCCGCCGACGGCGCCGGCCCCGACGAGGAACCGCCCGAGACCGTGCGC  73440
       P  Y  G  R  P  A  D  G  A  G  P  D  E  E  P  P  E  T  V  R

73441  GACTTCGCCGCCCTGGTGGCCCTGGCCGACCGGCACCCGCGCCTCGTCGACGTCGACGGC  73500
       D  F  A  A  L  V  A  L  A  D  R  H  P  R  L  V  D  V  D  G

73501  AGGAAGCTCAACGCCACCCTCGCCCACTACATCCAGCTGGGCCTGGAACGCCAGCTGACC  73560
       R  K  L  N  A  T  L  A  H  Y  I  Q  L  G  L  E  R  Q  L  T

73561  GCCGTCTTCGCCGAGCTCGCCCCGCTGTGCGCCCGCAACGGCATCGCACCGGACATCTGC  73620
       A  V  F  A  E  L  A  P  L  C  A  R  N  G  I  A  P  D  I  C

73621  CTCTCCGGCGGTACCGCCCTCAACGCCATCGCCACCCAACTCGCCTTCGAGTCGACCGGC  73680
       L  S  G  G  T  A  L  N  A  I  A  T  Q  L  A  F  E  S  T  G

73681  TTCGAGCGCATGCACCTCCACCCCGCCTGCGGCGACGACGGCACCGCGATCGGCGCGGCG  73740
       F  E  R  M  H  L  H  P  A  C  G  D  D  G  T  A  I  G  A  A

73741  CTCTGGCACTGGCACCACGTCCTGGGCCACCCCCGGCTCCACCACACCAACGCCGACCTC  73800
       L  W  H  W  H  H  V  L  G  H  P  R  L  H  H  T  N  A  D  L

73801  ATGTACTCCGTCCGTGAGTACCCCGAGCACACCGTCCGGCGGGCCGTGCGGGACCACGCG  73860
       M  Y  S  V  R  E  Y  P  E  H  T  V  R  R  A  V  R  D  H  A

73861  GCCGACCTCGTCGTCGAGGAGACCGGCGACTACGTCGCCAGGGCCGCCGAACTGGTCGCC  73920
       A  D  L  V  V  E  E  T  G  D  Y  V  A  R  A  A  E  L  V  A

73921  GGCGGCGCCGTCATCGGCTGGTACGACGGCGCCGGCGAGGTCGGGCCGCGGGCCCTGGGC  73980
       G  G  A  V  I  G  W  Y  D  G  A  G  E  V  G  P  R  A  L  G

73981  CACCGCAGCATCGTCGCCGACCCGCGCGACCCCGCCATGCGGGACCGGCTCAACTCCCAG  74040
       H  R  S  I  V  A  D  P  R  D  P  A  M  R  D  R  L  N  S  Q

74041  GTCAAGTTCCGCGAACACTTCCGGCCcTTCGCGCCGTCCGTGCTCAAGGAGCACGCCGCG  74100
       V  K  F  R  E  H  F  R  P  F  A  P  S  V  L  K  E  H  A  A

74101  GAGTGGTTCGGCCTCTCCGACAGCCCCTTCATGCTGCGGGCCACCCCCGTCCTCAAGCCC  74160
       E  W  F  G  L  S  D  S  P  F  M  L  R  A  T  P  V  L  K  P

74161  GGCGTGCCCCGCCATCACCCACGTCGACGGGACGTCGAGGATCCAGTCGGTCACCCGCCAG  74220
       G  V  P  A  I  T  H  V  D  G  T  S  R  I  Q  S  V  T  R  Q

74221  GACACCCCCGCCTTCCACGACCTCATCCACGCCTTCAAGGACCGTACGGGGATCCCCATG  74280
       D  T  P  A  F  H  D  L  I  H  A  F  K  D  R  T  G  I  P  M

74281  GTGCTCAACACCAGCCTCAACACCAAGGGCGAGCCGATCGCGGAGACACCCGAGGACGCC  74340
       V  L  N  T  S  L  N  T  K  G  E  P  I  A  E  T  P  E  D  A

74341  CTGCGCACCCTGCTCGGCTCCCGGCTCGACCACCTGGTGCTCCCGGGCCTCATCGTCAGC  74400
       L  R  T  L  L  G  S  R  L  D  H  L  V  L  P  G  L  I  V  S

74401  GGCCGGACGGCGGCCCGCTCATGAGCGCCCCGCGGGGCGAGCGGACCCGGCGCCGCGCGC  74460  (orf10)
                              M  S  A  P  R  G  E  R  T  R  R  R  A  L
       G  R  T  A  A  R  S  *

74461  TCGAACGCGACATCGCCGCGATCTGGGCCGAGACCCTCGGCAGGGACAGCGTCGGCCCGC  74520
         E  R  D  I  A  A  I  W  A  E  T  L  G  R  D  S  V  G  P  H

74521  ACGAGGACTTCGCCGCGCTGGGCGGCAACTCCATCCACGCCATCAAGATCACCAACCGGG  74580
         E  D  F  A  A  L  G  G  N  S  I  H  A  I  K  I  T  N  R  V

74581  TGGAGGAACTCGTCGACGCCGAGCTGTCCATCCGCGTCCTGCTCGAGACGCGCACCGTGG  74640
         E  E  L  V  D  A  E  L  S  I  R  V  L  L  E  T  R  T  V  A

74641  CCGGCATGACGGACCACGTCCACGCCACGCTCACGGGGGAGCGGGACCGGTGAACACCGA  74700  (orf9)
         G  M  T  D  H  V  H  A  T  L  T  G  E  R  D  R  *
                                                        M  N  T  D
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74701  CCTGCCCCGGCTGCTCGACCGGATCGCCGGCCTGCGCGTCCTCGTCATCGGCGACGTCAT  74760
         L  P  R  L  L  D  R  I  A  G  L  R  V  L  V  I  G  D  V  I

74761  CCTCGACACCTACGTCTGGGGAGCCACCTCGGGCCTGTGCCGCGAATCCCCCGTCCCTGC  74820
         L  D  T  Y  V  W  G  A  T  S  G  L  C  R  E  S  P  V  P  A

74821  CGTCACCCTGACCTCCGTCGCCCACCAGTGCGGCGGCGCCGCCAACGTCGCCGTGAACCT  74880
         V  T  L  T  S  V  A  H  Q  C  G  G  A  A  N  V  A  V  N  L

74881  CCGGGCGCTCGGCGCCGAACCGGTGCTGCTCTCCGCGACGGGTGACGACCGCGCCGGCCG  74940
         R  A  L  G  A  E  P  V  L  L  S  A  T  G  D  D  R  A  G  R

74941  CCGGCTGCGCGAAGCCCTCCGTGCGCGGGACGTCGACACCGGCGGACTCTTCGTACAGCC  75000
         R  L  R  E  A  L  R  A  R  D  V  D  T  G  G  L  F  V  Q  P

75001  CGGCCGGACCACGGTCACCAAACGCCGCGTCATGGCCGACGGACAGATGCTGCTCCGCCT  75060
         G  R  T  T  V  T  K  R  R  V  M  A  D  G  Q  M  L  L  R  L

75061  CGACGAGGGCGGCGAACACCCGTTGCCCGTGGCGACGGACACCGGAAGCCGCCTGCTCGA  75120
         D  E  G  G  E  H  P  L  P  V  A  T  D  T  G  S  R  L  L  E

75121  ACGGGCCGCCGGCCTGCTGCCCGCCGTCGACGCCGTGATCGTCTCCGACTACGGGTACGG  75180
         R  A  A  G  L  L  P  A  V  D  A  V  I  V  S  D  Y  G  Y  G

75181  CGTGTGGGAGCCCGACACCGTCGCCCGGCTCGCCGCACACCGCGAACTCGGCCCGTCCAC  75240
         V  W  E  P  D  T  V  A  R  L  A  A  H  R  E  L  G  P  S  T

75241  CCTGGTCGTCGACTCCCGCCGGCCCGCGCGCTTCACCGCGCTGCGGGCCAGCGCCGTCAA  75300
         L  V  V  D  S  R  R  P  A  R  F  T  A  L  R  A  S  A  V  K

75301  ACCCAACCACGCGGAGGCGCTGCGCCTGCTCGACGCCGGCGAACCCCCGCCCGGCCCGGC  75360
         P  N  H  A  E  A  L  R  L  L  D  A  G  E  P  P  P  G  P  A

75361  CAGGGCGGACTGGGCGGCCGCCCTCGGCGACCGGCTCCTGCGCCTGACGGGAGCCGAACG  75420
         R  A  D  W  A  A  A  L  G  D  R  L  L  R  L  T  G  A  E  R

75421  GGTCGCCCTCACCCTGGACGCCGACGGATCACTGCTCTTCGAACGCGACCGGCCCCCGGT  75480
         V  A  L  T  L  D  A  D  G  S  L  L  F  E  R  D  R  P  P  V

75481  CCGCACGTTCGCCCGGGGCAGCCGGGCACCGGTCACGGCCGCCGTCGGCGCCGGCGACGC  75540
         R  T  F  A  R  G  S  R  A  P  V  T  A  A  V  G  A  G  D  A

75541  CTTCACCGCGGCCCTCACCCTCGCCCTCGCCGCCGGCGCCGACTCCGCGGTCGCCGCCGA  75600
         F  T  A  A  L  T  L  A  L  A  A  G  A  D  S  A  V  A  A  E

75601  ACTGGCCTCCGCCGCCGCCGGCACGGCCGTCGCCACCCCCGGCACCAGCACCTGGCACGC  75660
         L  A  S  A  A  A  G  T  A  V  A  T  P  G  T  S  T  W  H  A

75661  CGACGAACTGCGCCGACTGCTCGGCGGCACCGGCAAGGTCTGCCGGACCGGCACCCTGCC  75720
         D  E  L  R  R  L  L  G  G  T  G  K  V  C  R  T  G  T  L  P

75721  CGCCCGGCTGCTCGACCCGGCCGCCCGCGACCGCCGGGTCGTCTTCACCAACGGCTGCTT  75780
         A  R  L  L  D  P  A  A  R  D  R  R  V  V  F  T  N  G  C  F

75781  CGACCTCCTGCACGGCGGCCACGTCTCCTGCCTGAGCCGGGCCAAGGAACTGGGCGACCT  75840
         D  L  L  H  G  G  H  V  S  C  L  S  R  A  K  E  L  G  D  L

75841  GCTCGTCGTCGGCGTCAACTCCGACGCGAGCGTCCGACGCCTCAAGGGCCCCCGTCGCCC  75900
         L  V  V  G  V  N  S  D  A  S  V  R  R  L  K  G  P  R  R  P

75901  GGTGATCCCCCTCGCCGAACGCATGCGCGTCCTCGCCGCCCTGAGCTGCGTGGACCTCGT  75960
         V  I  P  L  A  E  R  M  R  V  L  A  A  L  S  C  V  D  L  V

75961  CGTGCCCTTCGACGACGACAGCCCCGCCGCCCTCATCGAGGCCCTCCGCCCCGAGGTCTA  76020
         V  P  F  D  D  D  S  P  A  A  L  I  E  A  L  R  P  E  V  Y

76021  CGCCAAGGGCGGGGACTACACCCTCGCGACCCTGCCCGAAGCACCCCTCGTCCAACGGCT  76080
         A  K  G  G  D  Y  T  L  A  T  L  P  E  A  P  L  V  Q  R  L

76081  CGGCGGCGTCGTCCACCTGCTCCCCAGCGTCGCCGACACCTCCACCACCGACATCATCCG  76140
         G  G  V  V  H  L  L  P  S  V  A  D  T  S  T  T  D  I  I  R

76141  GCGCATCCACGCCCTGTCCAGGACCGGCGAGGGAGACACCCCATGAGCCACGCCATCGGA  76200
                                                  M  S  H  A  I  G  (orf8)
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        R  I  H  A  L  S  R  T  G  E  G  D  T  P  *

76201   CCGAGCCGGCTGATCCCCGCCATCCGCGAAGCGCTCGGGGACGAGAAGGACCCCCGGCTC   76260
        P  S  R  L  I  P  A  I  R  E  A  L  G  D  E  K  D  P  R  L

76261   GCCCTCTACGTCCACGTCCCCTTCTGCTCCTCCAAGTGCCACTTCTGCGACTGGGTCACC   76320
        A  L  Y  V  H  V  P  F  C  S  S  K  C  H  F  C  D  W  V  T

76321   GACATCCCCGTCGCACGCCTGCGCGGCGACAGCCGGGAACGCTCGCCCTACGTCACCGCC   76380
        D  I  P  V  A  R  L  R  G  D  S  R  E  R  S  P  Y  V  T  A

76381   CTCTGCGACCAGATCCGCTTCTACGGCCCCCAGCTCACCCGGCTCGGCTACCGCCCCGAG   76440
        L  C  D  Q  I  R  F  Y  G  P  Q  L  T  R  L  G  Y  R  P  E

76441   GTCATGTACTGGGGCGGCGGCACCCCCACCCGGCTCACCGGCGACGAGATGACGGCCGTC   76500
        V  M  Y  W  G  G  G  T  P  T  R  L  T  G  D  E  M  T  A  V

76501   CACCAGGCCCTCGACGACGCCTTCGACCTGACGGGACTCCGCCAGTGGTCGGTGGAGAGC   76560
        H  Q  A  L  D  D  A  F  D  L  T  G  L  R  Q  W  S  V  E  S

76561   ACCCCGAACGACCTCGACCCCGCCACCCTCGACACCCTGCGCGGCCTCGGCGTCACCCGC   76620
        T  P  N  D  L  D  P  A  T  L  D  T  L  R  G  L  G  V  T  R

76621   GTCAGCGTCGGCGTCCAGTCGCTCAACCCGTACCAGCTGCGCAAGGCAGGCCGGGCCCAC   76680
        V  S  V  G  V  Q  S  L  N  P  Y  Q  L  R  K  A  G  R  A  H

76681   TCGCGCGAACAGGCCCTGGCCGCCGTCCCCCTGTTGCGCCGCGCCGGCATCGACGAGTTC   76740
        S  R  E  Q  A  L  A  A  V  P  L  L  R  R  A  G  I  D  E  F

76741   AACGTCGACCTGATCGCCGGCTTCCCCGGCGAAGCCGTCGAGTCCTTCGAGGAGACCCTG   76800
        N  V  D  L  I  A  G  F  P  G  E  A  V  E  S  F  E  E  T  L

76801   CGCACCGTCCTCGCGCTCGACCCGCCGCACGTCTCCGTCTACCCCTACCGCGCCACCCCC   76860
        R  T  V  L  A  L  D  P  P  H  V  S  V  Y  P  Y  R  A  T  P

76861   AAGACGGTCATGGCCATGCAGCTCGACCGCGAGTTCGTCGAGGCCCGGAACCGGGACGGC   76920
        K  T  V  M  A  M  Q  L  D  R  E  F  V  E  A  R  N  R  D  G

76921   ATGATCGACGCCTATGAACGGGCCATGGCCGCGCTCGGCGCCGCCGGCTATCACGAGTAC   76980
        M  I  D  A  Y  E  R  A  M  A  A  L  G  A  A  G  Y  H  E  Y

76981   TGCCACGGCTACTGGGTGCGCGACGCGCGCCACGAGGACCAGGACGGCAACTACAAGTAC   77040
        C  H  G  Y  W  V  R  D  A  R  H  E  D  Q  D  G  N  Y  K  Y

77041   GACCTGGCCGGCGACAAGATCGGCTTTGGCAGCGGCGCCGAATCGATCATCGGTCACCAC   77100
        D  L  A  G  D  K  I  G  F  G  S  G  A  E  S  I  I  G  H  H

77101   CTGCTCTGGAACGAGAACAGCGCCTACGCCCGCTACCTGCTCGCCCCCCGCGAGTTCTCC   77160
        L  L  W  N  E  N  S  A  Y  A  R  Y  L  L  A  P  R  E  F  S

77161   GCCGCCCACCGGTTCACCACCGCCGAACCCGACCGCCTGACCGCCCCCGTCGGCGGCGCG   77220
        A  A  H  R  F  T  T  A  E  P  D  R  L  T  A  P  V  G  G  A

77221   CTGATGACCCGTGAAGGCGTGGTCTTCGCCCGCTTCCGCAGACTGACCGGCCTGGACTTC   77280
        L  M  T  R  E  G  V  V  F  A  R  F  R  R  L  T  G  L  D  F

77281   GCGGACGTCCGCGCCACACCGTACTTCCGCCAGTGGTTCGAGCTCCTGGAGCGCTGCGGC   77340
        A  D  V  R  A  T  P  Y  F  R  Q  W  F  E  L  L  E  R  C  G

77341   GGCCGCTTCGTCGAGACGCCGTACAGCCTCCGCCTGGAGCCGTCCACCATCCACCGCGCC   77400
        G  R  F  V  E  T  P  Y  S  L  R  L  E  P  S  T  I  H  R  A

77401   TACATCACCCACCTCGCCTACACCATGGCCCATGGCCTGGCCCCCGAACGCGCCTGA      77457
        Y  I  T  H  L  A  Y  T  M  A  H  G  L  A  P  E  R  A  *
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SEQ ID NO: 2 ORFS BLM gene cluster ORFs 31-40
(notice this part is on the reverse strand and the last nucleotide (18660) is the first (1) on the whole cluster of 77457 bp. Also the last orf (40) is incomplete and contains frame shifts)

```
1    GTGACCGAGAACCTTCCGTCGTGCCCCGAATGCTCCAGCGCGTACACCTATGAGATGGGT   60
     M  T  E  N  L  P  S  C  P  E  C  S  S  A  Y  T  Y  E  M  G
     (orf31)

61   GCGCTCCTGGTCTGCCCCGAATGCGGCCACGAGTGGCCGCCCGCGACCGCCGAGTCCGCG  120
     A  L  L  V  C  P  E  C  G  H  E  W  P  P  A  T  A  E  S  A

121  GACAACCCCGAAGACGGCGCGATCAGGGACGCGGTCGGCAACGTACTCGCCGACGGCGAC  180
     D  N  P  E  D  G  A  I  R  D  A  V  G  N  V  L  A  D  G  D

181  ACCGTCACGGTGGTCAAGAGCCTGAAGGTCAAGGGCCACCCGACCGGCATCAAGGCCGGC  240
     T  V  T  V  V  K  S  L  K  V  K  G  H  P  T  G  I  K  A  G

241  ACCAAGGTGCGCAACATCCGCCTCGTGGAGGGTGTGGCCGGCCACGACATCGACTGCAAG  300
     T  K  V  R  N  I  R  L  V  E  G  V  A  G  H  D  I  D  C  K

301  ATCGACGGGTTCGGCGCCATGCAGCTCAAGTCCAGCGTGGTCAAGAAGGTCTGACCGGTT  360
     I  D  G  F  G  A  M  Q  L  K  S  S  V  V  K  K  V  *

361  ACGCCGGCCCAGGCCCTGCCCAGGCTCCACTACGCCGCGGCGCAACCGAGCCGGAACGGG  420

421  GCCCGGGCCCGCTCCAAGTCCCGTTCCGTGCGCGGCCGCGGCAGCCAGGCCGTGTTCACC  480

481  CTGGGGTCGCCGTCCCCGTTCGCACGCGTCGTACACGCCACCACGCACGGCACGGAACTC  540

541  CCCGAACTCGCCACGTTCCCCAAGTCCCCGCGTGCCCGGATCCGCCCGGACCGGCGTCGG  600

601  TCCGCCCGCCGGGCCGCGGCCGGGTCCCCGGGCCGCGGCGGGAGGGGGTCTCGCGCCGTG  660

661  GAACGCCGGCCGGAAATTTACGTATAGGTAGAGATCCCGGCGAAGCGATCGGCGCGTTAT  720

721  GGCAGCATCCGCGCCGGCCCGCCGCGCAGTTCCTCGGTCCCGGACCGATGGCGTCAAAAG  780

781  TGAGCGACGAAATCGCCGGATCGCGCGAGGACCGTCGCGGGCCGCACGAGGACAACCGGG  840

841  GGATATATCAGCGCATTCCCAGGTCACGCGTTGACTGGAAATCGCCTACTTATCGCGTCA  900

901  CGCCTGTAGGGATCATGGCCGGGAATGGCCTCAGACGCTTTGAGTGCCCACCTTGAGGTT  960
                              M  A  S  D  A  L  S  A  H  L  E  V
                              (orf32)

961  TCCGACTGTCGGCAGCGCGGGGGGATCACGGTGACGAATGACGGATCTGAACTCGCCGGG  1020
     S  D  C  R  Q  R  G  G  I  T  V  T  N  D  G  S  E  L  A  G

1021 CAAAACGTGGCGGCGGTCCGCTTCGAGCGGTATTCCGCGATCGCGCCGGAGCGGACCGCC  1080
     Q  N  V  A  A  V  R  F  E  R  Y  S  A  I  A  P  E  R  T  A

1081 ATCCTGCACAAAGGTGCCGCGACCGGTTACGACGAGCTCAACCGCCGGGCCGAGCTGACA  1140
     I  L  H  K  G  A  A  T  G  Y  D  E  L  N  R  R  A  E  L  T

1141 GCCACGCGCCTGGCGGACGCGGGCGCCGGCCCCTCGACCCTGGTGGCAGTGGCCCTCCCA  1200
     A  T  R  L  A  D  A  G  A  G  P  S  T  L  V  A  V  A  L  P

1201 CGCGATCCCGACCTCGTCGCCACCCTGTGCGCCCTGCTCAAACTGGGTGCCGCATGCCTT  1260
     R  D  P  D  L  V  A  T  L  C  A  L  L  K  L  G  A  A  C  L

1261 CCCCTGGATCCCGGCATACCGGCCGGGCGGCTGCGCGAGATCATGGCCGACGCGTCCCCC  1320
     P  L  D  P  G  I  P  A  G  R  L  R  E  I  M  A  D  A  S  P

1321 GACGTTCTCGTCACCACCCGTGCCGTCGCTCCGGCATTCACCGGTGACGGACCCGTCCTT  1380
     D  V  L  V  T  T  R  A  V  A  P  A  F  T  G  D  G  P  V  L
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1381 TTCCTGGACGACGCTCCTCCGACCTGCTCCGCCGTCCTTCCACGGCACTCAGCGGGGACC 1440
     F  L  D  D  A  P  P  T  C  S  A  V  L  P  R  H  S  A  G  T

1441 GCGTCGGAAATCGCCTATGTGCTGTACCCGACGACTCCTGACGAGAAGTCCGAAAATTCG 1500
     A  S  E  I  A  Y  V  L  Y  P  T  T  P  D  E  K  S  E  N  S

1501 GTCGTCTCCTATCGTGATATGGCGCGCTACCTTGACGACCCCACTGCCGGGATTCCGGCG 1560
     V  V  S  Y  R  D  M  A  R  Y  L  D  D  P  T  A  G  I  P  A

1561 AGGGCGGAGATTCTCCGGCTGGTCGCGCCGCTCCTGTCCGGCGGTCGTCTGGTGCTGGAC 1620
     R  A  E  I  L  R  L  V  A  P  L  L  S  G  G  R  L  V  L  D

1621 GCCGACGAGACCCGGCCCCGGCCGGTCACCCGTGAGGCGCCGCGCGACATGGTGGAGGAC 1680
     A  D  E  T  R  P  R  P  V  T  R  E  A  P  R  D  M  V  E  D

1681 GTCGTGGCGCAGGTCTGGTGCGCCGTGCTCGGCGTGGACCGGGTGGGCGTGCGGGACCGC 1740
     V  V  A  Q  V  W  C  A  V  L  G  V  D  R  V  G  V  R  D  R

1741 TTCTTCGACCTGGGCGGCAAGTCGCTGGCGGCGGTCCAGGTGGTGGCGCGCCTGCGGAAG 1800
     F  F  D  L  G  G  K  S  L  A  A  V  Q  V  V  A  R  L  R  K

1801 CTGCTCGGCGTCGAGCTGCCGCTGCGGGCCCTGTTCGACGCGCCGACGGTCGAGGAGCTG 1860
     L  L  G  V  E  L  P  L  R  A  L  F  D  A  P  T  V  E  E  L

1861 GCCGCCCGGGTGCGGGCCGAACAGGCCGGCGGCCAGGGCGTCCGGGAGGAGGCGGCGCTC 1920
     A  A  R  V  R  A  E  Q  A  G  G  Q  G  V  R  E  E  A  A  L

1921 GAGCCGGTGGGCCGGAGCGAGCCGCTGCCGCTGTCGTTCGCACAGCAACGCCTGTGGTTC 1980
     E  P  V  G  R  S  E  P  L  P  L  S  F  A  Q  Q  R  L  W  F

1981 CTGGACCGCTTGATGCCCGACCGCGCCTTCTACACGATGTGCGACGCGTTCCGCGTCCGG 2040
     L  D  R  L  M  P  D  R  A  F  Y  T  M  C  D  A  F  R  V  R

2041 GGCGGGATCGACCTGGGTGCGCTGCGGCGGGCCCTGCGGATGCTGGTGGGACGGCACGAG 2100
     G  G  I  D  L  G  A  L  R  R  A  L  R  M  L  V  G  R  H  E

2101 ACGCTGCGGACGGCGTTCGTCGAGCGGGACGGTGTGCCGTACCAGCTCGTCGGTCCGGCC 2160
     T  L  R  T  A  F  V  E  R  D  G  V  P  Y  Q  L  V  G  P  A

2161 GACGGGCCCGGTGCGCGGCGCGTGGCCGCTCCCACGCGGGTCGACCTGTCGCTGCTGGAG 2220
     D  G  P  G  A  R  R  V  A  A  P  T  R  V  D  L  S  L  L  E

2221 CCCGCCGAGCGGGAGGAGGCGGTGCGGAACCTGGTGGCGGCGGAGGCGCGGACCCCGTTC 2280
     P  A  E  R  E  E  A  V  R  N  L  V  A  A  E  A  R  T  P  F

2281 CGGCCGGCGGACGGCGCGCTGCTGCGCGTGGTGGTGGCCCGGCTGGCGGACGATGATCAC 2340
     R  P  A  D  G  A  L  L  R  V  V  V  A  R  L  A  D  D  D  H

2341 GTGCTGGTGGTCAGCACGCACCACATCGTCTCCGACGCCTGGTCCGTGGGTGTGCTGGTG 2400
     V  L  V  V  S  T  H  H  I  V  S  D  A  W  S  V  G  V  L  V

2401 GACGAACTCGGACGGCTGTACCGCGAGTGCGTCACCGGAGATCCCGCCGCGCTGCCCCCG 2460
     D  E  L  G  R  L  Y  R  E  C  V  T  G  D  P  A  A  L  P  P

2461 CCGGCCGTCCAGTACGCCGACTTCGCGGTCTGGCAGCGGGCCTGGATGGCCGGTCCGGTG 2520
     P  A  V  Q  Y  A  D  F  A  V  W  Q  R  A  W  M  A  G  P  V

2521 CAGGAGGAGCATCTCGCGTACTGGAAGCGGGCCTTGGACGGCGCTCCCTCGGTGCTGCGG 2580
     Q  E  E  H  L  A  Y  W  K  R  A  L  D  G  A  P  S  V  L  R

2581 CTGCCCATGGACCACCCGCGGCCCGCCGTGCAGTCCGAGCGGGGCGAGACGGTCGGGTTC 2640
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      L  P  M  D  H  P  R  P  A  V  Q  S  E  R  G  E  T  V  G  F

2641  GCGCTGCCCGACGCGCTGGTCGCCGCGCTGGAGAAGCTGGGCCGGGAGCAGGGCGCCACC  2700
      A  L  P  D  A  L  V  A  A  L  E  K  L  G  R  E  Q  G  A  T

2701  CTGTTCATGACGCTGCTCGGCGCCTTCCAGGTCCTGCTGGCGCGTCACGCCGGGCAAGAG  2760
      L  F  M  T  L  L  G  A  F  Q  V  L  L  A  R  H  A  G  Q  E

2761  GACATCGTGGTCGGCGTGCCGGCGGCGGGGCGCACCCGGACCGAGACGGAACCTCTGGTC  2820
      D  I  V  V  G  V  P  A  A  G  R  T  R  T  E  T  E  P  L  V

2821  GGCTTCTTCGTCAACACGCTTCCCTTGCGGGCGATCTGCGCTCCGGGCCTGTCGTTCCGG  2880
      G  F  F  V  N  T  L  P  L  R  A  I  C  A  P  G  L  S  F  R

2881  GACCTGCTGGACCAGGTGCGCGAGGCCGCCCTCGGCGCCTTCGCCCATCAGGACCTCCCC  2940
      D  L  L  D  Q  V  R  E  A  A  L  G  A  F  A  H  Q  D  L  P

2941  TTCGAGGCGCTGGTCGAGGCGCTCGCACCCGAGCGCGACCTCGGCCACAATCCCCTCGTC  3000
      F  E  A  L  V  E  A  L  A  P  E  R  D  L  G  H  N  P  L  V

3001  CAGGTCACCTTCCAGCTCCTGGGCACACCGGCGGCGCGGCCGGACCTGATCGGGACGGAG  3060
      Q  V  T  F  Q  L  L  G  T  P  A  A  R  P  D  L  I  G  T  E

3061  GTCGAGCGGTACCCGGTCCAGGAGGCCGTCTCGCAGTTCGACCTGTCCCTGGACATCAAG  3120
      V  E  R  Y  P  V  Q  E  A  V  S  Q  F  D  L  S  L  D  I  K

3121  CGGGCCGACGACGGTTCCTACCGGGGGATCCTGAACTACTGCCCCGACCTGTTCGACCGA  3180
      R  A  D  D  G  S  Y  R  G  I  L  N  Y  C  P  D  L  F  D  R

3181  CGCCGCATGGAGGTGCTGGTCGGCCACTACCTGACGCTGCTCGGCGCCGCCGCCGCGGAC  3240
      R  R  M  E  V  L  V  G  H  Y  L  T  L  L  G  A  A  A  A  D

3241  CCGGGCCGCCCGATCGGTGAGCTGCCGCTGTCCGACGGGGCCGAACGGCTGCGGCTGCTC  3300
      P  G  R  P  I  G  E  L  P  L  S  D  G  A  E  R  L  R  L  L

3301  GACGGGTTCGGGAAGCGGGACGCGGCGTACGCCGGGCCGGGAAGCGTTCCGGAGCGGTTC  3360
      D  G  F  G  K  R  D  A  A  Y  A  G  P  G  S  V  P  E  R  F

3361  GCGGAGGTGGCGCGGACGGCACCGGACGCGCGGGCGGTGACGTGTGGCGCGACAACGCTC  3420
      A  E  V  A  R  T  A  P  D  A  R  A  V  T  C  G  A  T  T  L

3421  ACCTTCGCCGAGCTGAACGACCGGGTGGAGCGCCTGGCACAGGCACTGCTCGGCGCCGGG  3480
      T  F  A  E  L  N  D  R  V  E  R  L  A  Q  A  L  L  G  A  G

3481  GTCACCCGCGAGACGCCGGTCGCGGTCCGCCTGCCCCGTTCCACCGACAGCGTCGTCGCC  3540
      V  T  R  E  T  P  V  A  V  R  L  P  R  S  T  D  S  V  V  A

3541  CTGCTGGCCGTCATGCGGGCGGGCGGCGTCTACGTCCCCCTGGACCCCGACTGGCCCGCG  3600
      L  L  A  V  M  R  A  G  G  V  Y  V  P  L  D  P  D  W  P  A

3601  GACCGCACCGCCTACATCCTGGACGACACCGCGGCCTCCGTCGTCATCACCCGCGACCTG  3660
      D  R  T  A  Y  I  L  D  D  T  A  A  S  V  V  I  T  R  D  L

3661  CCCGCACTCCCCGGTCGCCTCCACGTCGACCCGCGCCGGCCCGCGGCCGACGGCCTGGTA  3720
      P  A  L  P  G  R  L  H  V  D  P  R  R  P  A  A  D  G  L  V

3721  CCCGCGCCCCGCATCGACCCCGATCAGGCCGCGTACGTCATCTACACGTCCGGCTCGACG  3780
      P  A  P  R  I  D  P  D  Q  A  A  Y  V  I  Y  T  S  G  S  T

3781  GGCGCGCCGAAGGGCGTCGTCGTCCGGCACCGCTCCCTGAACCACCTCACCAGCGCCCTG  3840
      G  A  P  K  G  V  V  V  R  H  R  S  L  N  H  L  T  S  A  L
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3841 CAGGCCACCTTTCTCGGCCACGACCCGTATCTCGCCGGGGCCGACGGCGTACCGCCCGGG 3900
     Q  A  T  F  L  G  H  D  P  Y  L  A  G  A  D  G  V  P  P  G

3901 GACGCGAAGCTGCGTACGACGCTCACCGCGCCCTTCACGTTCGACGCGTCCATGGAGCAA 3960
     D  A  K  L  R  T  T  L  T  A  P  F  T  F  D  A  S  M  E  Q

3961 CTGAGCTGGATGCTGGCCGGTCACGAGCTGTTCATCGTGCCCGAGGACGTGCGGCGCGAC 4020
     L  S  W  M  L  A  G  H  E  L  F  I  V  P  E  D  V  R  R  D

4021 CCCTCGGCGCTGGTCCGGTTCGTCCGGGAGCACCGGATCGACGTCATCGACACGACCTCC 4080
     P  S  A  L  V  R  F  V  R  E  H  R  I  D  V  I  D  T  T  S

4081 TCGCAGCTCGAACTCCTCGTATCGCACGGGCTGTTGGACGGAGAGTGGGCGCCGTCCATG 4140
     S  Q  L  E  L  L  V  S  H  G  L  L  D  G  E  W  A  P  S  M

4141 GTCATGGTGGGTGGCGAGGCGGTCTCGCCGTCGCTGTGGCGGACCTTGCGGGACCAGCGG 4200
     V  M  V  G  G  E  A  V  S  P  S  L  W  R  T  L  R  D  Q  R

4201 CGCACTCGCTGTTTCAACCTGTACGGGCCTACGGAGGCGACGGTCGACGCCACCTGCCAC 4260
     R  T  R  C  F  N  L  Y  G  P  T  E  A  T  V  D  A  T  C  H

4261 GACCTGTCCGACCCCGCCGACGTCCCCGTCATCGGCACCCCACTCCCCCACACCCACGTC 4320
     D  L  S  D  P  A  D  V  P  V  I  G  T  P  L  P  H  T  H  V

4321 CGCGTGCTCGACGACCGACTGCGACCCGTACCCGTGGGCGTCGCCGGCGAGATCTACCTC 4380
     R  V  L  D  D  R  L  R  P  V  P  V  G  V  A  G  E  I  Y  L

4381 GGCGGAACCGGCCTGGCCCGCGGCTACCTCAACCGCCCCGCCCTCACCGCCCGACGCTTC 4440
     G  G  T  G  L  A  R  G  Y  L  N  R  P  A  L  T  A  R  R  F

4441 GTCGCCGACCCCTACCCCGACACCCCCGGCAGCCGCCTGTACCGCACCGGCGACCGCGCC 4500
     V  A  D  P  Y  P  D  T  P  G  S  R  L  Y  R  T  G  D  R  A

4501 CGCTGGCGCCCCGACGGCACCCTCGAATACCTGGGACGCACCGACGACCAAATCAAGATC 4560
     R  W  R  P  D  G  T  L  E  Y  L  G  R  T  D  D  Q  I  K  I

4561 CGCGGCTTCCGCGTCGAACCCGGCGAAATCGAGGCCGTCCTCACCCACCACCCCGCCGTC 4620
     R  G  F  R  V  E  P  G  E  I  E  A  V  L  T  H  H  P  A  V

4621 AAGGAAGCCGCCGTCGTCGACGACGCGCACGCGCGGCTGGTCGCCTACGTCACGCTCGCG 4680
     K  E  A  A  V  V  D  D  A  H  A  R  L  V  A  Y  V  T  L  A

4681 GAAGGCGGCGGCGCCGGCCCCACCGACGTACGCCGGTTCGCGCAGGGGCGGCTGCCCGCC 4740
     E  G  G  G  A  G  P  T  D  V  R  R  F  A  Q  G  R  L  P  A

4741 CACATGGTGCCGTCGGCGGTGGTCGTCCTGGAGGCGCTGCCACTGACGTCGAACGGAAAG 4800
     H  M  V  P  S  A  V  V  V  L  E  A  L  P  L  T  S  N  G  K

4801 CTGGACCGCGCGCGCCTGCCGGCGCCCGCGGCGGGCAGACCGGAACTGGATGTCCGCTTC 4860
     L  D  R  A  R  L  P  A  P  A  A  G  R  P  E  L  D  V  R  F

4861 GTGGCGCCGCGCGACATGGTGGAGGAGGTCGTGGCGCAGGTCTGGTGCGCCGTGCTGGGC 4920
     V  A  P  R  D  M  V  E  E  V  V  A  Q  V  W  C  A  V  L  G

4921 GTCGACCGGGTCGGTGTGCACGACGACTTCTTCGAGCTGGGCGGGCACTCGTTGCTGGTG 4980
     V  D  R  V  G  V  H  D  D  F  F  E  L  G  G  H  S  L  L  V

4981 GTCCAGGTGATGACCCGGATACGAAAGCTGCTCGGCGTCGAGGTGCCGTTGCGGGAGCTG 5040
     V  Q  V  M  T  R  I  R  K  L  L  G  V  E  V  P  L  R  E  L

5041 TTCGACGCCGCGACGGTCGAGGAGCTCGCCGCCCGCGTCCGCGCCGCACGGACCGAGGGC 5100
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      F  D  A  A  T  V  E  E  L  A  A  R  V  R  A  A  R  T  E  G

5101  CTCGGCCGGGGGGCCGCCCCGCCCCTCGGGCCGGTGGACCGGAGCGGGCCGCTGCCGCTG  5160
      L  G  R  G  A  A  P  P  L  G  P  V  D  R  S  G  P  L  P  L

5161  TCGTTCGCGCAGCAACGCCTTTGGTACCTCGATCAGTTGGCGCCCGACAGTGTCTCCTAC  5220
      S  F  A  Q  Q  R  L  W  Y  L  D  Q  L  A  P  D  S  V  S  Y

5221  AACATGTGCGACGCCTACCGGGTCCGCGGCCCTCTCGACCTGGACGCGCTGCGGCGGGCG  5280
      N  M  C  D  A  Y  R  V  R  G  P  L  D  L  D  A  L  R  R  A

5281  CTGCGGACGCTGGTCGAGCGGCACGAGACGCTGCGGACGGCGTTCGTCGAGCGGGACGGG  5340
      L  R  T  L  V  E  R  H  E  T  L  R  T  A  F  V  E  R  D  G

5341  GTGCCCCACCAGGTGGTCTCGGCGCCCGACGCGCCGGCCGCGCGGCGCGCGGCGGAGGTC  5400
      V  P  H  Q  V  V  S  A  P  D  A  P  A  A  R  R  A  A  E  V

5401  GTGCGGATCGAGGCGGCCGGGCGGACCGACGAGGCGGTGCGGGACCTGGTGGCCGCGGAG  5460
      V  R  I  E  A  A  G  R  T  D  E  A  V  R  D  L  V  A  A  E

5461  GCGCGCACCCCGTTCCGGCCGGCGGACGGCGCGCTGATGCGCGTGGCGGTGGCCCGGCTG  5520
      A  R  T  P  F  R  P  A  D  G  A  L  M  R  V  A  V  A  R  L

5521  GCGGACGACGATCACGTGCTGGTGGTCACCACGCACCACATCGTCTCCGACGGCTGGTCG  5580
      A  D  D  D  H  V  L  V  V  T  T  H  H  I  V  S  D  G  W  S

5581  GTCGACATCCTGGTGGACGAATTGGGGCGCCTGTACCGGGAACACGTCACGGGTGACCCC  5640
      V  D  I  L  V  D  E  L  G  R  L  Y  R  E  H  V  T  G  D  P

5641  GCCGGGCTCCCTCCGCTCGACGTCCAGTACGCCGACTTCGCCGTCTGGCAGCGGTCCTGG  5700
      A  G  L  P  P  L  D  V  Q  Y  A  D  F  A  V  W  Q  R  S  W

5701  ATGACCGGCCCCGTGCGGGAGGAGCACCTCGCGTACTGGAAGCGGGCCCTGGACGGGGCA  5760
      M  T  G  P  V  R  E  E  H  L  A  Y  W  K  R  A  L  D  G  A

5761  CCCTCGGTCCTGCGGCTGCCGGCGGACCATCCGCGTCCCGCCGTCCAGTCCCAGCGGGGC  5820
      P  S  V  L  R  L  P  A  D  H  P  R  P  A  V  Q  S  Q  R  G

5821  GAGACCGTCGAGTTCCCCCTGCCCGCACCACTGGTCGCGCGGCTGGAAGCGCTCTGCCGG  5880
      E  T  V  E  F  P  L  P  A  P  L  V  A  R  L  E  A  L  C  R

5881  GAGCAGGGCGTCACCCTGTTCATGGCGCTCTTCGGCGCGTTCCAGGTGTTGCTGGCGCGC  5940
      E  Q  G  V  T  L  F  M  A  L  F  G  A  F  Q  V  L  L  A  R

5941  TACAGCGGTCAGGACGACGTGGTCGTGGGCGTGCCGACGGCGAACCGCACCCGCGCGGAG  6000
      Y  S  G  Q  D  D  V  V  V  G  V  P  T  A  N  R  T  R  A  E

6001  ACCGAGCCCCTGGTCGGCTTCTTCGTCAACACCCTTCCGGTACGGGTCGCGTGCTCGCCG  6060
      T  E  P  L  V  G  F  F  V  N  T  L  P  V  R  V  A  C  S  P

6061  GAGCTGTCGTTCCGCGCCCTGCTCGACCGGGTCCGCGAGGCCGCGCTGGGCGCCTTCGCC  6120
      E  L  S  F  R  A  L  L  D  R  V  R  E  A  A  L  G  A  F  A

6121  CATCAGGACCTGCCCTTCGAGGCGCTGGTCGAGGCGCTCGCGCCCGAGCGCGACCTGGGC  6180
      H  Q  D  L  P  F  E  A  L  V  E  A  L  A  P  E  R  D  L  G

6181  CACCACCCTCTCGTGCAGGTCACCTTCCAACTCCTCGACGCTCCCGACGAGAGGCTCGTC  6240
      H  H  P  L  V  Q  V  T  F  Q  L  L  D  A  P  D  E  R  L  V

6241  CTGCACGGCACGGACTGCGTCTCGCTCGGCTTCGGCGGTGTGACCAGCCGGTTCGACCTG  6300
      L  H  G  T  D  C  V  S  L  G  F  G  G  V  T  S  R  F  D  L
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6301 TCCCTCGACGTCGTCTCGGGGCGGCGGGGGAAGCGGTGCGTGCTGACGTACTGTCCCGAC 6360
     S  L  D  V  V  S  G  R  R  G  K  R  C  V  L  T  Y  C  P  D

6361 CTGTTCGACCGGCCCCGCATGGAGGTGCTGGCCGGCCACTACCTGACCCTGCTCGGCGCG 6420
     L  F  D  R  P  R  M  E  V  L  A  G  H  Y  L  T  L  L  G  A

6421 GCGGCCGACGATCCCGGTCTCCGCGTCGGCGACCTCCCGCTGAGCGACGACGTCGAACGC 6480
     A  A  D  D  P  G  L  R  V  G  D  L  P  L  S  D  D  V  E  R

6481 CTGCGCCTGCTGGGCGGGTCCCGCCCGCGGTACCTGCCCGCGCCCGGGGCGGAGACCGTC 6540
     L  R  L  L  G  G  S  R  P  R  Y  L  P  A  P  G  A  E  T  V

6541 CCTGACGCCTTCGCCGCGCAGGTGCGGGCGACACCGGACGCGCCCGCGCTGGTCCACGGG 6600
     P  D  A  F  A  A  Q  V  R  A  T  P  D  A  P  A  L  V  H  G

6601 GACTCGACGCTGACGTTCGCCGAGCTGGACACCCGGGTCACCGCCCTGGCCGTGCGGTTG 6660
     D  S  T  L  T  F  A  E  L  D  T  R  V  T  A  L  A  V  R  L

6661 CGGCGCTGCGGCGTGGCCGCCGAGACGCCGGTCGCGGTGTGCCTGCCGCGCTCCGCCGAC 6720
     R  R  C  G  V  A  A  E  T  P  V  A  V  C  L  P  R  S  A  D

6721 GCCGTCGTGGCCCTCCTGGCCGTCCTGCGGGCGGGCGGCGTCTATGTGCCAGTGGATCCG 6780
     A  V  V  A  L  L  A  V  L  R  A  G  G  V  Y  V  P  V  D  P

6781 GAGTGGCCCTCCGGCCGCGTCGCCCACGTCCTCGACGAGACCGCGGCCCCCGTCGTCATC 6840
     E  W  P  S  G  R  V  A  H  V  L  D  E  T  A  A  P  V  V  I

6841 ACCCGCGACCTGCCCGCCGATCCCGGCCGCGTCCACCTCGACCCGCGCCAGGCCCCGGCC 6900
     T  R  D  L  P  A  D  P  G  R  V  H  L  D  P  R  Q  A  P  A

6901 GACGACCGGGATCCCCTGCCGCGCCTCCACCGCGACCAGGCCGCGTACATCATCTTCACC 6960
     D  D  R  D  P  L  P  R  L  H  R  D  Q  A  A  Y  I  I  F  T

6961 TCGGGCTCCACCGGCGCCCCCAAGGGCGTCGTCGTCCGACACGGCTCCCTGTACCACCTC 7020
     S  G  S  T  G  A  P  K  G  V  V  V  R  H  G  S  L  Y  H  L

7021 CTGGGCCACGTACGGCGCATGGCGGAGGGCGGCCCCCGGCGGAACGTCGCGCACACCACC 7080
     L  G  H  V  R  R  M  A  E  G  G  P  R  R  N  V  A  H  T  T

7081 GCGATGACCTTCGACCCGTCGCTGGAACAGTTCCTGTGGCTCGTCGCCGGACACACCCTG 7140
     A  M  T  F  D  P  S  L  E  Q  F  L  W  L  V  A  G  H  T  L

7141 CACGTCGCGCCCGAGGAGGTGCGCCGCGATCCCGAGGCGCTGGTGGCCCTGGTGCGGCGC 7200
     H  V  A  P  E  E  V  R  R  D  P  E  A  L  V  A  L  V  R  R

7201 GCCGCGATCGACGTCCTCAACGTCACCCCGTCCCACCTGACCCTGCTGATCGAGGCCGGG 7260
     A  A  I  D  V  L  N  V  T  P  S  H  L  T  L  L  I  E  A  G

7261 CTGCTGGAGGGCGACCGGGTGCCGGGTACGGTCCTGGTGGGTGGCGAGGCGGTGCCCGCG 7320
     L  L  E  G  D  R  V  P  G  T  V  L  V  G  G  E  A  V  P  A

7321 GCGCTGTGGCGGACCCTGCGCGAACGGACGGGAGCCACCCGCTTCTTCAACCTGTACGGG 7380
     A  L  W  R  T  L  R  E  R  T  G  A  T  R  F  F  N  L  Y  G

7381 CCTACGGAGGCGACGGTCGACGCCACCTGCCACGACCTGTCCGACCCCGCCGACGTCCCC 7440
     P  T  E  A  T  V  D  A  T  C  H  D  L  S  D  P  A  D  V  P

7441 GTCATCGGCACCCCACTCCCCCACACCCACGTCCGCGTGCTCGACGACCGACTGCGACCC 7500
     V  I  G  T  P  L  P  H  T  H  V  R  V  L  D  D  R  L  R  P

7501 GTACCCGTGGGCGTCGCCGGCGAAATCTACCTCGGCGGAACCGGCCTGGCCCGCGGCTAC 7560
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      V  P  V  G  V  A  G  E  I  Y  L  G  G  T  G  L  A  R  G  Y

7561  CTCAACCGCCCCGCCCTCACCGCCCAACGCTTCGTCGCCGACCCCTACCCCGACACCCCC  7620
      L  N  R  P  A  L  T  A  Q  R  F  V  A  D  P  Y  P  D  T  P

7621  GGCAGCCGCCTGTACCGCACCGGCGACCGCGCCCGCTGGCGCCCCGACGGCACCCTCGAA  7680
      G  S  R  L  Y  R  T  G  D  R  A  R  W  R  P  D  G  T  L  E

7681  TACCTGGGACGCACCGACGACCAAATCAAGATCCGCGGCTTCCGCGTCGAACCCGGCGAG  7740
      Y  L  G  R  T  D  D  Q  I  K  I  R  G  F  R  V  E  P  G  E

7741  ATCGAAGCCGTCCTCACCCACCACCCCGCCGTCAAGGAAGCCGCCGTCACCGTGGCCACC  7800
      I  E  A  V  L  T  H  H  P  A  V  K  E  A  A  V  T  V  A  T

7801  GACGACGGTGCCGCCCGGCTGGTCGCCCTCGTCGTCCCCGCCCCCCGCGCCCCGCACGGC  7860
      D  D  G  A  A  R  L  V  A  L  V  V  P  A  P  R  A  P  H  G

7861  GATTCGGCCGACGGCGCCCCGGACGCCCAGGTCGAGGAGTGGAACGCCGTCTTCGAGGCG  7920
      D  S  A  D  G  A  P  D  A  Q  V  E  E  W  N  A  V  F  E  A

7921  ACCCACACCGACGCCGCCGACGGCGAACTCACCTTCAACATCAAGGGCTGGAACGACAGC  7980
      T  H  T  D  A  A  D  G  E  L  T  F  N  I  K  G  W  N  D  S

7981  CTCACCGGTGCGCCGATCCCCGCCGAACACATGCGGGAATGGGTCGACACCACCGTCGCC  8040
      L  T  G  A  P  I  P  A  E  H  M  R  E  W  V  D  T  T  V  A

8041  CGGCTCCTGGAACGGCCGGCCGAGCGCGTCCTGGAGATCGGCAGTGGCACCGGGCTGCTG  8100
      R  L  L  E  R  P  A  E  R  V  L  E  I  G  S  G  T  G  L  L

8101  ATGTGGCGGCTGCTGCCGCACGTCACCGAGTACACCGGAACCGACTTCTCGCGGCCCGCC  8160
      M  W  R  L  L  P  H  V  T  E  Y  T  G  T  D  F  S  R  P  A

8161  GTGGACTGGCTCCGGGACGGGCTGCGCCGCCGCCCCGCGCACCGGGTACGGCTGCTGCAC  8220
      V  D  W  L  R  D  G  L  R  R  R  P  A  H  R  V  R  L  L  H

8221  CGCGAGGCGACCGACTTCACCGGCGTCCGCGCCGCGTCCACCGACCTCGTCGTCGTCAAC  8280
      R  E  A  T  D  F  T  G  V  R  A  A  S  T  D  L  V  V  V  N

8281  TCGGTCGTCCAGTACTTCCCCGACCGCGCCTACCTCGACACCGTCCTGGCCCGCGCCCTC  8340
      S  V  V  Q  Y  F  P  D  R  A  Y  L  D  T  V  L  A  R  A  L

8341  GACGCCACGGCCGACCGAGGGCGCGTCTTCGTGGGCGACGTGCGCAACCTGGCCCTCGCC  8400
      D  A  T  A  D  R  G  R  V  F  V  G  D  V  R  N  L  A  L  A

8401  CCGCAGTTCTACGCCCGTCAGGCCCTCGCCCACGCCGGTCCGGGCGCGGCGGCGCGGGAC  8460
      P  Q  F  Y  A  R  Q  A  L  A  H  A  G  P  G  A  A  A  R  D

8461  GTGGCGCGCGCCGCCGGCGAGTTCGCGGCCATGGACGGCGAGTTGCTGGTGTCCCCCGCG  8520
      V  A  R  A  A  G  E  F  A  A  M  D  G  E  L  L  V  S  P  A

8521  TACTTCGCCGCGCTCGCCGCCCGCTCGCCCCGCGTCACCGGCGTCGAGATCCTGCCCCGC  8580
      Y  F  A  A  L  A  A  R  S  P  R  V  T  G  V  E  I  L  P  R

8581  CGGGGACGGCACCGCAACGAGATGAGCCTGTACCGCTACGACGTGGTGCTGCACGTGGGC  8640
      R  G  R  H  R  N  E  M  S  L  Y  R  Y  D  V  V  L  H  V  G

8641  GGTGACCGCCCGGCGGCCCCGGAGGCGGAGGTGCTCACCTGGGGCGACCAGGTGCACGAC  8700
      G  D  R  P  A  A  P  E  A  E  V  L  T  W  G  D  Q  V  H  D

8701  CTCGCGTCGCTGTCCGCCCGCCTCGGCCGCGGGGGCCCGGACGCCCTGCTCGTGCGCGGC  8760
      L  A  S  L  S  A  R  L  G  R  G  G  P  D  A  L  L  V  R  G
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8761  GTCGCCAACGACCGTCTGACGCGGGACAACGAGCTGCTCGACGCACCCGCCCGCACGACG  8820
      V  A  N  D  R  L  T  R  D  N  E  L  L  D  A  P  A  R  T  T

8821  GCCGTCGAGCCCGAGGACCTGTGGGGGCTGGCGGACTCCACCCCCTACCGGGTGAGCGTC  8880
      A  V  E  P  E  D  L  W  G  L  A  D  S  T  P  Y  R  V  S  V

8881  AGCTGGGCCGCCGCCGATCCGCGGGGCGCGATGGACGTCCTGCTGGTCCGGCGGGACGCC  8940
      S  W  A  A  A  D  P  R  G  A  M  D  V  L  L  V  R  R  D  A

8941  CACGACGACGGTCCGCTGCTCGTCCCCCACCCCGTACCGGAGCCCTCGGCACCGCTGACG  9000
      H  D  D  G  P  L  L  V  P  H  P  V  P  E  P  S  A  P  L  T

9001  AACACGCCGACCCGGCACCCGTCCGCGCGGCAAGGGGGCTCGGCCGCGGACGGGCTGCGT  9060
      N  T  P  T  R  H  P  S  A  R  Q  G  G  S  A  A  D  G  L  R

9061  TCCTGGCTCGCCGAGCGGCTTCCCGCGCACCTGCTGCCCGCGAGGATCACCGAGGTGGAC  9120
      S  W  L  A  E  R  L  P  A  H  L  L  P  A  R  I  T  E  V  D

9121  GCGCTGCCCCCGCACCGGCACCGGCAAGCTCGACCGGGGCGCGCTCGGCGGACTCGTGACC  9180
      A  L  P  R  T  G  T  G  K  L  D  R  G  A  L  G  G  L  V  T

9181  GCGGGCCGTGGCGCCCGGGCGGGCGACCGCCCCGCCACCGCCCCCCGTACGGGTCTCGAA  9240
      A  G  R  G  A  R  A  G  D  R  P  A  T  A  P  R  T  G  L  E

9241  CGGACCCTGGCCGACGCGTGGGCGCGGGTGCTCGGCCTCCCCGAAGTCGGCGTGCACGAG  9300
      R  T  L  A  D  A  W  A  R  V  L  G  L  P  E  V  G  V  H  E

9301  AACTTCTTCGCCCTCGGCGGCGACTCCCTCCTCGCCGTCAGGGCTGTCGCCCGGTGCCGC  9360
      N  F  F  A  L  G  G  D  S  L  L  A  V  R  A  V  A  R  C  R

9361  CGTGCCGGGGTCCGACTGACCGTCCGGCAGTTGCTGAGCGAGCAGACCGTCGCCGCGCTC  9420
      R  A  G  V  R  L  T  V  R  Q  L  L  S  E  Q  T  V  A  A  L

9421  GCGGCGGCCCTCGAGGAGGAGTCTCAATGATGAAGTCAAGCCGCTTGCGCGACCGGCAGC  9480
      A  A  A  L  E  E  E  S  Q  *
                                M  M  K  S  S  R  L  R  D  R  Q  L
                                (orf33)

9481  TCGGGGGTGAAGACCCGGTTGTCGCGCAGGAGAGCCCACAGGACGCTGGCCCGACGCCGT  9540
        G  G  E  D  P  V  V  A  Q  E  S  P  Q  D  A  G  P  T  P  C

9541  GCCAGGGCGATGACGGCTTGAACGTGTTTGCAGCCCTCGCCGCGCTTCTTGAGGTAGAAG  9600
        Q  G  D  D  G  L  N  V  F  A  A  L  A  A  L  L  E  V  E  V

9601  TCCCGGTTCGGCCCCTCCCGCATCATGCTGGTTTGGGCCGACATGTAGAACACTCGTCGC  9660
        P  V  R  P  L  P  H  H  A  G  L  G  R  H  V  E  H  S  S  Q

9661  AGGCGGCGGCTGTAGCGCTTGGGCCGATGCAGGTTGCCAGTGCGACGACCGGAGTCGCGG  9720
        A  A  A  V  A  L  G  P  M  Q  V  A  S  A  T  T  G  V  A  G

9721  GGGACGGGCACCAGGCCGGCCGCCGAGGCCAGGTGACCGGCGTCGGCGTAGGCCGTGAGG  9780
        D  G  H  Q  A  G  R  R  G  Q  V  T  G  V  G  V  G  R  E  V

9781  TCGCCGGCGGCGACGACGAACTCGGCGCCGAGGATCGGCCCCATGCCCGGCAGAGACTCG  9840
        A  G  G  D  D  E  L  G  A  E  D  R  P  H  A  R  Q  R  L  D

9841  ATGATCTCGGCCTGTGGATGGCTGCGGAACGTCTCGCGGATCTGCTGGTCAATCCGCTTC  9900
        D  L  G  L  W  M  A  A  E  R  L  A  D  L  L  V  N  P  L  Q

9901  AGACGGTCGTCCAGGGCCAGGATCTGCGCGGCCAGGTCAGCCACGATCTGGGCGGCGACG  9960
        T  V  V  Q  G  Q  D  L  R  G  Q  V  S  H  D  L  G  G  D  V
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9961  TCCTCCCCGGGCAGCGCGGTCTGCTGAGCCTGGGCAGCCTCCAGCGCCGTCGCGGCGACG  10020
        L  P  G  Q  R  G  L  L  S  L  G  S  L  Q  R  R  R  G  D  G

10021 GCGTCGGCACCGCGCACGCCTCGGTTGGCCAGCCAGGCCGTCAGCCGGGCCCGGCCGCGG  10080
        V  G  T  A  H  A  S  V  G  Q  P  G  R  Q  P  G  P  A  A  A

10081 CGGCGGAGAGCTGCCGGGGTCTGGTAGCCCGTCAGCAGGACCAGCGCGCCCTTCTGCGAG  10140
        A  E  S  C  R  G  L  V  A  R  Q  Q  D  Q  R  A  L  L  R  A

10141 CTGTAGTCGAAGGCCCGTTCCAGCGCGGGGAAGACGCCGGTCAGCGTGTCGCGGAGACGG  10200
        V  V  E  G  P  F  Q  R  G  E  D  A  G  Q  R  V  A  E  T  V

10201 TTGATCATCCTGACCCGGTCGGCCACGAGGTCGGAACGGTGGGCGGTCAGCAGCGCGAGG  10260
        D  H  P  D  P  V  G  H  E  V  G  T  V  G  G  Q  Q  R  E  V

10261 TCGGCGGCCAGCTGGGCGGGCACGTCGATCGACGCGAAGTCCCGTCGGTTGCGGGCGGTT  10320
        G  G  Q  L  G  G  H  V  D  R  R  E  V  P  S  V  A  G  G  F

10321 TCGGCGATGACGTAGGCGTCGCGGGCGTCGGTCTTCGCCTCGCCCCGGTAAGCGCCGGAC  10380
        G  D  D  V  G  V  A  G  V  G  L  R  L  A  P  V  S  A  G  H

10381 ATGCGGTTGACCGTGCGGCCGGGCACGTAGACGGCCTGCTGGCCGTGGGCCGCGAGCAGG  10440
        A  V  D  R  A  A  G  H  V  D  G  L  L  A  V  G  R  E  Q  G

10441 GCCAGCAGCAGCGCGGAGGACGTGCCGGAGATGTCCACTGCCCAGTGGACCTCGTCGGCC  10500
        Q  Q  Q  R  G  G  R  A  G  D  V  H  C  P  V  D  L  V  G  Q

10501 AGGTCGAGGATCTCACCCATGGCGGTCAGGATCGCCGACTCATCGTTGCCGATCTTCTTC  10560
        V  E  D  L  T  H  G  G  Q  D  R  R  L  I  V  A  D  L  L  R

10561 GACCACAGCGTCACACCGGTCTCGTCGACCACCGCCGCCCAGTGATGCCCCTTGCCCGCG  10620
        P  Q  R  H  T  G  L  V  D  H  R  R  P  V  M  P  L  A  R  V

10621 TCGATCCCGGCCCAGACCCGGGCCCGTCGCTCGCCCACTCGCCCCTCCTCACTCCGAACA  10680
        D  P  G  P  D  P  G  P  S  L  A  H  S  P  L  L  T  P  N  S

10681 GCATCCCGTCGACCCGAGGAACACCCCGCTGTCATCTCCGTAAAAAGCGACCGAAGCGCA  10740
        I  P  S  T  R  G  T  P  R  C  H  L  R  K  K  R  P  K  R  T

10741 CATCTCAATCAGCAGCCAGGGCGCCCCGGAGAACCGGGCGGCCACTCCTTGTAAGCCACT  10800
        S  Q  S  A  A  R  A  P  R  R  T  G  R  P  L  L  V  S  H  *

10801 GACGGCAGAGAACCATAAGCCACACCCGGCCCTCCCGGGCCGCCTAACAACTTACGGAGA  10860

10861 ACCATGACTGACCTGCCGTTGCGTACCGTCGCACTCACCGGTGAGGAGAGCGCGGAGGTC  10920
          M  T  D  L  P  L  R  T  V  A  L  T  G  E  E  S  A  E  V
          (orf34)

10921 GACGACCTGCTGCGCACGCTGGCCGACGTGCCGGTCGACTCCACCGTGGGACTGCTGCAC  10980
        D  D  L  L  R  T  L  A  D  V  P  V  D  S  T  V  G  L  L  H

10981 CGCACCCGGCTCGCCGCACAGGAACTGCCGCTGCGCATCCGCGCCGAGCTCACGGGGATG  11040
        R  T  R  L  A  A  Q  E  L  P  L  R  I  R  A  E  L  T  G  M

11041 CGGCTCTACGACAGCCCGCGCGCCCTCGTCGTCACGGGCTTCGGCGTCGACGACGAACGG  11100
        R  L  Y  D  S  P  R  A  L  V  V  T  G  F  G  V  D  D  E  R

11101 ATCGGACCGACCCCCGCGGCCCGTCCCGCCCCGGATCCCGAGCGGACCCGCGACCTGGAG  11160
        I  G  P  T  P  A  A  R  P  A  P  D  P  E  R  T  R  D  L  E

11161 CTGCTGCTTTTGCTGCACGCGGCCCTGCTCGGCGAGGCGTTCGGCTGGGCGACCCAGCAG  11220
        L  L  L  L  L  H  A  A  L  L  G  E  A  F  G  W  A  T  Q  Q
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11221 AACGGCCGGCTCGTCCACGACGTGCTGCCCGTTCCCGGTGAGGAGACCGCGCAGATGGGT 11280
      N  G  R  L  V  H  D  V  L  P  V  P  G  E  E  T  A  Q  M  G

11281 TCCAGCAGCGAGACCGAGCTGCTGTGGCACACCGAGGACGCGTTCCACCCGCTGCGCTGC 11340
      S  S  S  E  T  E  L  L  W  H  T  E  D  A  F  H  P  L  R  C

11341 GACTACGTGGGCCTGCTGTGCCTGCGCAACCACCAGCGCGCCGCGACCACCGTGGGCTGG 11400
      D  Y  V  G  L  L  C  L  R  N  H  Q  R  A  A  T  T  V  G  W

11401 CCCGACCTGTCCCGGCTCACCACCGAGGACCGTGCCGTGCTCCTCGAACCCCGCTATCTG 11460
      P  D  L  S  R  L  T  T  E  D  R  A  V  L  L  E  P  R  Y  L

11461 ATCCGCCCGGACACCTCGCACACGCCCGCGCAGAACGCGACGGGCACGCGGTCCGCCGAG 11520
      I  R  P  D  T  S  H  T  P  A  Q  N  A  T  G  T  R  S  A  E

11521 CGTTTCGCGGCGATCGCCGAGATGGACGACGCCCCGGAGCGCGTCGCCGTCCTGTTCGGC 11580
      R  F  A  A  I  A  E  M  D  D  A  P  E  R  V  A  V  L  F  G

11581 GACCCCGAGGACCCGTACCTGCGGATCGACCCGGCCTACATGAGCCCGGCCCCCGGGGAC 11640
      D  P  E  D  P  Y  L  R  I  D  P  A  Y  M  S  P  A  P  G  D

11641 GCGGCCGCCCGGCGGGCGTACGACACCGTCACCGCGCTCATCGAGGACGAGCTGCGGCAC 11700
      A  A  A  R  R  A  Y  D  T  V  T  A  L  I  E  D  E  L  R  H

11701 GTCGTCCTGGACGCCGGTTCACTGCTGCTGGTCGACAACTACCAGGCGGTGCACGGCCGC 11760
      V  V  L  D  A  G  S  L  L  L  V  D  N  Y  Q  A  V  H  G  R

11761 AAGCCGTTCGCCGCCGCCTACGACGGCCGCGACCGCTGGCTCAAACGCGTCAACATCACC 11820
      K  P  F  A  A  A  Y  D  G  R  D  R  W  L  K  R  V  N  I  T

11821 CGCGACCTGCGCCGTTCCCGGTCCGCGCGGCGGTCGGCCACCTCGCTGCTGGTGTGAGGG 11880
      R  D  L  R  R  S  R  S  A  R  R  S  A  T  S  L  L  V  *

11881 AGGCACCATGGATTTCCCCCTCACCCGCGTCAACCCCTGGTTCAGCGGCGGCTGCGACGG 11940
            M  D  F  P  L  T  R  V  N  P  W  F  S  G  G  C  D  G
            (orf35)

11941 CCGCCCCCGGGTGCGGCTGTGCGCGCTGCCGTACGCGGGCGGCACCGCCGCCGTCTTCAA 12000
      R  P  R  V  R  L  C  A  L  P  Y  A  G  G  T  A  A  V  F  K

12001 GGACTGGCCCGCCGCGCTGCCCCCCGGAGTGGAGCTGCTCACCGCGCACCTGCCGGGACG 12060
      D  W  P  A  A  L  P  P  G  V  E  L  L  T  A  H  L  P  G  R

12061 CGGCGACCGGTTCACCGAACCGCCCCCGGCCACCCTGGAGGAGACCGCCGAGCGGCTGTG 12120
      G  D  R  F  T  E  P  P  P  A  T  L  E  E  T  A  E  R  L  C

12121 CGAGGCGCTGCCGCCGAGTGACCTGCCCACGGTCGTCCTCGGCCACAGCATGGGCGCCCT 12180
      E  A  L  P  P  S  D  L  P  T  V  V  L  G  H  S  M  G  A  L

12181 GCTGGGGTACGAAGTGGCGGCGCGGCTCGCGGCGCGGGGCCGCGCCCCCAACCTGCTGAT 12240
      L  G  Y  E  V  A  A  R  L  A  A  R  G  R  A  P  N  L  L  I

12241 CGCCGCGGCCTGCCGTCCCCCGCACGTTCCGCCGGACGCCTCCGGTCCGGTGACCGAGGC 12300
      A  A  A  C  R  P  P  H  V  P  P  D  A  S  G  P  V  T  E  A

12301 CGAGCTGGCGGCCACCCTGCGGGCCGAACGCCCATGGGACACGGCCCTGAGGGACGAGGA 12360
      E  L  A  A  T  L  R  A  E  R  P  W  D  T  A  L  R  D  E  E

12361 ACTGATGGAAGCGGTGCTGCCCGCCCTGGTCGCCGACATCACGGCCGGCGACCGCTACCA 12420
      L  M  E  A  V  L  P  A  L  V  A  D  I  T  A  G  D  R  Y  H

12421 CCGCCCGCGGCCCCGCCCGCTCGACCTCCCGCTGAAGGTCTACATCGGCGCCGACGACGA 12480
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        R  P  R  P  R  P  L  D  L  P  L  K  V  Y  I  G  A  D  D  D

12481   CGGCACCGACTGGCGCACCACCCTGGGCTGGCGCGCGTGCACCGCCCGGGACTGCGAGGT   12540
        G  T  D  W  R  T  T  L  G  W  R  A  C  T  A  R  D  C  E  V

12541   CGTCGTCCTGCCCGGCGGCCACTACTTCCTGGAGACCGACCGCGCGGCCGTCCTCACCCG   12600
        V  V  L  P  G  G  H  Y  F  L  E  T  D  R  A  A  V  L  T  R

12601   CGTCGCCACGGACCTCGCCGAAGCCGAGGTAGGGGCATGACCGCGCGCGTCGACGCCACA   12660
        V  A  T  D  L  A  E  A  E  V  G  A  *
                                          M  T  A  R  V  D  A  T
                                          (orf36)

12661   CCCACCTACCTGGCGGTGCTGGCGGTGCGCGAGGCCCGCGCCCCGCTCCTCGGCAGCTGC   12720
        P  T  Y  L  A  V  L  A  V  R  E  A  R  A  P  L  L  G  S  C

12721   CTGGCCCGCATGTCCTTCGCGGTGCTGCCGCTCGCCCTGCTGCTGTCGGTCCGGGACGCG   12780
        L  A  R  M  S  F  A  V  L  P  L  A  L  L  L  S  V  R  D  A

12781   ACGGGGTCGTTCGCCGTCGCCGGACTGACCTCCGGCGCGCTGTCGGCCACGCTCACGCTG   12840
        T  G  S  F  A  V  A  G  L  T  S  G  A  L  S  A  T  L  T  L

12841   TTCGCGCCCGCCCGCGCCCGGCTGATCGACCGCCGGGGCTCACGGTCCGGACTGGTCCGG   12900
        F  A  P  A  R  A  R  L  I  D  R  R  G  S  R  S  G  L  V  R

12901   CTGACCGTCCCGTACCTGCTGGGGCTCGCCGTGCTGATCACATTGGCCGAGGCGGAAGCG   12960
        L  T  V  P  Y  L  L  G  L  A  V  L  I  T  L  A  E  A  E  A

12961   CCCACCGCGGCGCTGCTCGTCGCCGCCGCGGTCGCGGGCGTGTTCGCGCCGCCGCTCGGT   13020
        P  T  A  A  L  L  V  A  A  A  V  A  G  V  F  A  P  P  L  G

13021   CCGACCATGCGCGTGCTGTGGGCGAGGATCCTGCACGGCCGTCAGCCCCTGCTGCACACC   13080
        P  T  M  R  V  L  W  A  R  I  L  H  G  R  Q  P  L  L  H  T

13081   GCCTACGCCCTCGACTCCGTCACCGAGGAGGTGGTCTTCACCGTGGGGCCGCTGCTGGCG   13140
        A  Y  A  L  D  S  V  T  E  E  V  V  F  T  V  G  P  L  L  A

13141   GGCGGCCTGATCGCGGTCGCGGCACCGCTCGCGTCGATGATCACGGTCATGGTGCTGATC   13200
        G  G  L  I  A  V  A  A  P  L  A  S  M  I  T  V  M  V  L  I

13201   GCGGCCGGTACCGCCTGCTTCGTGCTGTCCGCCGCGACCGCCGCCGCCCCCGCGTCGGGC   13260
        A  A  G  T  A  C  F  V  L  S  A  A  T  A  A  A  P  A  S  G

13261   GAAGCCGACGAGGACCGGCCGCACGGCCGGCCCATGGCTCTGCCCGGGATGCGCACGATC   13320
        E  A  D  E  D  R  P  H  G  R  P  M  A  L  P  G  M  R  T  I

13321   GTGCTGTCCTTCGGCGGCGTCGGCCTGGTCGTCGGGGTGCTCCAGGTCGTCCTGCCGTTC   13380
        V  L  S  F  G  G  V  G  L  V  V  G  V  L  Q  V  V  L  P  F

13381   ATCGCCGACCACGCGGGCTCGCCCGGCGCGGGCGGCATCCTGCTGTCCATGCTGTCGGCG   13440
        I  A  D  H  A  G  S  P  G  A  G  G  I  L  L  S  M  L  S  A

13441   GGCAGCGCGGTCGGCGGCCTCGCCTACGGGCGGATCGCCTGGCGCTCGACGCCCGTGCGG   13500
        G  S  A  V  G  G  L  A  Y  G  R  I  A  W  R  S  T  P  V  R

13501   CGGTTCGTGGTGCTCGTCACCGGGTTCACGCTGGCGGTGCTGCCGCTGTGCCTGACCGCG   13560
        R  F  V  V  L  V  T  G  F  T  L  A  V  L  P  L  C  L  T  A

13561   AGCCCGGTGCCGGCCGGGGCCTTCGCCCTCCTCGTGGGACTCTGCCTCGCCCCGCTGTTC   13620
        S  P  V  P  A  G  A  F  A  L  L  V  G  L  C  L  A  P  L  F

13621   ACCACCGCCTACCTGCTGGTCAACGACCTGGTGACGGCGTCGGGGACCGCACCCACCGAG   13680
        T  T  A  Y  L  L  V  N  D  L  V  T  A  S  G  T  A  P  T  E
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13681 GCCAACACCTGGGTCTCCACGGCCAATAACGGAGGGTTCGCCGCGGGCAGCGCCGCCGCC 13740
      A  N  T  W  V  S  T  A  N  N  G  G  F  A  A  G  S  A  A  A

13741 GGTGTGCTGCTCGACTCCCGGGGCCCCACCGTCACCGTCACCGCCGCGTTCGCGGTCGCC 13800
      G  V  L  L  D  S  R  G  P  T  V  T  V  T  A  A  F  A  V  A

13801 GCCGCGACCGCCGTCATGACCGTTCTGCGCCGCCGGACCCTGCTCCTCGGCGCCGGACAC 13860
      A  A  T  A  V  M  T  V  L  R  R  R  T  L  L  L  G  A  G  H

13861 CCCGAACCGGCCGCCGCCACACCCGCCGACCGCACCGCACCCGCCGAAGCCGAGGAGTGA 13920
      P  E  P  A  A  A  T  P  A  D  R  T  A  P  A  E  A  E  E  *

13921 ACCGATCGTGTCCAAGAACGCGGCGCACTGGTCGCGCATCCGCACAGGGGACGCCCCCGG 13980
            M  S  K  N  A  A  H  W  S  R  I  R  T  G  D  A  P  G
            (orf37)

13981 CGTCGTACTCGCCGTGGACTTCTACGGAACGGGCCGCCAGGAAGCCACCTTCCGCCACCT 14040
      V  V  L  A  V  D  F  Y  G  T  G  R  Q  E  A  T  F  R  H  L

14041 GTGCGACCTGCTCACGGATCCGGTCGAGGTCTGGCACGCGGTCCCGCCCGCCCCGGACGG 14100
      C  D  L  L  T  D  P  V  E  V  W  H  A  V  P  P  A  P  D  G

14101 CGACTGGTCCACGGCCACCGGCGCCGGTCACCTGCGCTGGTGGACCGAGGGGCTCGACAC 14160
      D  W  S  T  A  T  G  A  G  H  L  R  W  W  T  E  G  L  D  T

14161 GGTCCTCGCGGGACGGCCGGTGCGGGCCCTCGTCGGCTACTGCGCGGGCGGCGTCTTCGC 14220
      V  L  A  G  R  P  V  R  A  L  V  G  Y  C  A  G  G  V  F  A

14221 CTCGGCCCTCGCCGACGCCCTCGTCGAACGGGAGGGCCACCGGCCGCGGGTCGTGCTGTT 14280
      S  A  L  A  D  A  L  V  E  R  E  G  H  R  P  R  V  V  L  F

14281 CAACCCCAGCGCGCCCGGCGTCGCCACGCTCACCCGCGACTTCCGCGGTCTGATCGCCGG 14340
      N  P  S  A  P  G  V  A  T  L  T  R  D  F  R  G  L  I  A  G

14341 CATGGACCTCCTCACGGACGGGGAACGCGCCGCTCTGCTGGCCGAGACGACCGCGATCCG 14400
      M  D  L  L  T  D  G  E  R  A  A  L  L  A  E  T  T  A  I  R

14401 GCGGGCACACGCCCCCGACGCGCTGGTACCGGTCGCCGAACGCTACGCCGCCCTGTACCG 14460
      R  A  H  A  P  D  A  L  V  P  V  A  E  R  Y  A  A  L  Y  R

14461 CGAGGGCTGCGACCTCCTGTGCGAGCGGCTCGGCGTGGACGCCTCCTTCGGCGCCGAACT 14520
      E  G  C  D  L  L  C  E  R  L  G  V  D  A  S  F  G  A  E  L

14521 GGCCGCCGTCCTCCACTCCTACCTGGCCTACCTCACGGCGGCGCTCGACGTGCCCCCCAC 14580
      A  A  V  L  H  S  Y  L  A  Y  L  T  A  A  L  D  V  P  P  T

14581 CCCGCTGTGGCGCGGCGCCGTCTCGCTCACCTCCCGCGAGCACCAGGGCACCGACTTCAC 14640
      P  L  W  R  G  A  V  S  L  T  S  R  E  H  Q  G  T  D  F  T

14641 CGACGTCGAGCACGGCTTCGACGTCGCCCGTGCCGAACTGCTGAGCTCCCCCCAGGTCGT 14700
      D  V  E  H  G  F  D  V  A  R  A  E  L  L  S  S  P  Q  V  V

14701 CGCGGCGCTGACCGCGCTCCTCCGCGAACACGAGGCGAGCCGATGACCCTCACCCTGCGG 14760
      A  A  L  T  A  L  L  R  E  H  E  A  S  R  *
                                                M  T  L  T  L  R
                                                (orf38)

14761 GACGCCTTCCTCGACCAGGCCGCCCGGACCCCCGACGCCCACGCCGTCGTACACGGCGAC 14820
      D  A  F  L  D  Q  A  A  R  T  P  D  A  H  A  V  V  H  G  D

14821 ACTGTATGGACGTACCGCGAACTGGAACTGCGGGCCGGCCGCATGGCCCGGACGCTGGCC 14880
      T  V  W  T  Y  R  E  L  E  L  R  A  G  R  M  A  R  T  L  A
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14881 GCACGCGGCGCGGGCCCCGGCACGCTGGTGGCGGTACGCCTGCCGCGCGGTCCCGAACCG 14940
      A R G A G P G T L V A V R L P R G P E P

14941 GTCGCCGCGCTCCTCGCGGTCGTGCTGACGGGAGCGGGCTACGTGCCGCTCGCCGACGAC 15000
      V A A L L A V V L T G A G Y V P L A D D

15001 GACCCGCCGGACCGGTGCCGGCACATCCTCGACGACTGCGCCGCCGCGCTGCTGCTGGCC 15060
      D P P D R C R H I L D D C A A A L L L A

15061 GAGCACCCCTCGCGGGACGGACGCACCCTCACCCCGGACGAGGCGCTGGCACCCGCCCGC 15120
      E H P S R D G R T L T P D E A L A P A R

15121 CCGTTCGACGCGGCCCCGGTGCGGGCCGGCGACCCGGCGTACGTGATCTACACCTCCGGC 15180
      P F D A A P V R A G D P A Y V I Y T S G

15181 TCCAGTGGCCGTCCGAAGGGCGTGCTGGTCGAACAGGGCGCGCTCGGCGCCTACCTGGCA 15240
      S S G R P K G V L V E Q G A L G A Y L A

15241 CAGGCCCGCGCGCGCTACGACGGGCTGTCCGGACGGACGGTGCTGCACTCCTCGCTGTCC 15300
      Q A R A R Y D G L S G R T V L H S S L S

15301 TTCGACATGGCCGTGACCAGTCTGTGGGGCCCGCTCGTCAGCGGCGGCGCGATCCACGTG 15360
      F D M A V T S L W G P L V S G G A I H V

15361 CTCGACCTGAAGGCGATCGCCTCCGGCACCCAGCCGCCGCCCGCCGCCTCGGCACGTCCG 15420
      L D L K A I A S G T Q P P P A A S A R P

15421 TCCTTCCTCAAGGTCACTCCGTCCCACCTGCCGCTGCTGGGCCTGCTGCCGGACTCCTGC 15480
      S F L K V T P S H L P L L G L L P D S C

15481 CTGCCCACCGGGCAACTCGTGATCGGCGGCGAGGCGCTGACCGGCTCCGCGCTCGGACCC 15540
      L P T G Q L V I G G E A L T G S A L G P

15541 TGGCGCGCCGCGCACCCCGACGTCACGGTCGTCAACGAGTACGGGCCCACCGAGGCGACC 15600
      W R A A H P D V T V V N E Y G P T E A T

15601 GTCGGCTGCTGCGCGTACACCGTCCGCCCCGGTGACGCCGTGGACCCGGGTGCCGTCCCC 15660
      V G C C A Y T V R P G D A V D P G A V P

15661 ATCGGACGGCCGTTCGCGGGCACCCGCCTGTACGTGCTCGACGCGGACGGCGAGCCGGTC 15720
      I G R P F A G T R L Y V L D A D G E P V

15721 GCCGTGGGCGGTGTGGGTGAACTGCACATCGCGGGCGACCAGTTGGCGCGCGGATACCTG 15780
      A V G G V G E L H I A G D Q L A R G Y L

15781 GGGCGCCCGCGGCTGACCGAGGAACGCTTCGTCCCGGACCCGTTCGCCGCCGACGGCTCC 15840
      G R P R L T E E R F V P D P F A A D G S

15841 CGGATGTACCGCACCGGCGACCTGGTGCGCGAACGCCCGGACGGCGACCTGGAGTACCTC 15900
      R M Y R T G D L V R E R P D G D L E Y L

15901 GGGCGCGCGGACGGGCAGGTGAAGGTCTCCGGGTACCGGATCGAGCCCGGCGAGATCGAG 15960
      G R A D G Q V K V S G Y R I E P G E I E

15961 GCCGTGCTCCGCGGCCACGCGGGGGTGAGGGACTGCGCGGTCGTCGCCGTCGGCGAGGCG 16020
      A V L R G H A G V R D C A V V A V G E A

16021 GACGCCCGCCGGCTCGTCGCCTACGTGGTACCGGACCCGGACTCCCCGCCCGGCACCGCC 16080
      D A R R L V A Y V V P D P D S P P G T A

16081 GCGCCGGCGCGGCACGCGGCCGAGGCGCTGCCGCCGTACATGGTGCCGGCGACGTTCGTC 16140
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      A  P  A  R  H  A  A  E  A  L  P  P  Y  M  V  P  A  T  F  V

16141 ACCGTGCCCGAACTGCCGCTCACCCCCAACGGGAAGCTCGACCGGGACGCGCTGCCCGGC 16200
      T  V  P  E  L  P  L  T  P  N  G  K  L  D  R  D  A  L  P  G

16201 CCCCCTGCCGGCGACGCCGGGCCGGGCGACCGCACCCCGGCCGAGACCCTGCTGTGCGAG 16260
      P  P  A  G  D  A  G  P  G  D  R  T  P  A  E  T  L  L  C  E

16261 CTGCTGGCACGGGCCCTGGGCATCCCGGAGATCGACGCCGACGCCGACTTCCTGACGTCC 16320
      L  L  A  R  A  L  G  I  P  E  I  D  A  D  A  D  F  L  T  S

16321 GGCGGCACCAGCATCACCGCGCTGAAGCTGGTCGCCGGCGCCCGCCGGGTCGGCATCCGC 16380
      G  G  T  S  I  T  A  L  K  L  V  A  G  A  R  R  V  G  I  R

16381 CTCGAACTCACCACCGTCCTGCGCGAACGCACGGTGCGCCGCATCCTGGCGGCCCAGCCC 16440
      L  E  L  T  T  V  L  R  E  R  T  V  R  R  I  L  A  A  Q  P

16441 GACGCCGCCTCGCCCCTCGCCGAAGGAGTGCCCGAGTGACCGGTTCCGTAACGCTCACCC 16500
                                                  M  T  G  S  V  T  L  T  P
      D  A  A  S  P  L  A  E  G  V  P  E  *  (orf39)

16501 CCCTCGGCGGGATCATCCCCAGGCCCCGCGGCGAGGGGCTCACCACCGGCGCCGAGTACG 16560
        L  G  G  I  I  P  R  P  R  G  E  G  L  T  T  G  A  E  Y  D

16561 ACCTGGGGCCGCTCGGCGACGCGGGCCCCGACTGgGTGCGGGCCCACGGCCCGCGACTGC 16620
        L  G  P  L  G  D  A  G  P  D  W  V  R  A  H  G  P  R  L  R

16621 GCGAGCGCCTCGCCACCGACGGGCTGATCCTGCTGCACGGTCTGCCCACCGACGGAGACG 16680
        E  R  L  A  T  D  G  L  I  L  L  H  G  L  P  T  D  G  D  G

16681 GCGTCGACGGCTTCCACGACGTCGTCGGCTCCGTCGGCGGCGACCCGCTGCCCTACACCG 16740
        V  D  G  F  H  D  V  V  G  S  V  G  G  D  P  L  P  Y  T  E

16741 AGCGCTCCACCCCGCGCAGCGTGGTCAAGGGCAACATCTACACCTCGACCGAGTACCCGG 16800
        R  S  T  P  R  S  V  V  K  G  N  I  Y  T  S  T  E  Y  P  A

16801 CCGACCAGCCCATCCCGATGCACAACGAGAACTCCTACGCCGCCCATTGGCCGTCCACGC 16860
        D  Q  P  I  P  M  H  N  E  N  S  Y  A  A  H  W  P  S  T  L

16861 TCTACTTCTTCTGCCACACCGCGCCGGACACCGGCGGGGCCACGCCGATCGCCGACGGCC 16920
        Y  F  F  C  H  T  A  P  D  T  G  G  A  T  P  I  A  D  G  R

16921 GCGCCGTCCTCGACCTCATCCCGGCCGAGGTCAGGCGGCGGTTCTCCCAAGGGGTCGTCT 16980
        A  V  L  D  L  I  P  A  E  V  R  R  R  F  S  Q  G  V  V  Y

16981 ACACCCGTACGTTCCGCGCCGACATGGGACTGAGCTGGCAGGAAGCGTTCCAGACCGAGG 17040
        T  R  T  P  R  A  D  M  G  L  S  W  Q  E  A  F  Q  T  E  D

17041 ACCGCGGCGACGTCGAACGCCATTGCCGCGCCCACGGCCAGGAGTTCTCCTGGGACGGCG 17100
        R  G  D  V  E  R  H  C  R  A  H  G  Q  E  F  S  W  D  G  D

17101 ACGTCCTGCGCACCCGCCACCACCGCCCGGCGACCGCCGTCGACCCCGGCACCGGAGCCG 17160
        V  L  R  T  R  H  H  R  P  A  T  A  V  D  P  G  T  G  A  E

17161 AGGTGTGGTTCAACCAGGCGCACCTGTTCCACCCGTCCAGCCTGGATCCCGACCTGCGCC 17220
        V  W  F  N  Q  A  H  L  F  H  P  S  S  L  D  P  D  L  R  Q

17221 AGGTGCTCCTGGAGACGTACGGCGAGAACGGCCTGCCCCGCGACGCCCTGTTCGCCGACG 17280
        V  L  L  E  T  Y  G  E  N  G  L  P  R  D  A  L  F  A  D  G

17281 GCACCCCGATCCCCGACGCCGACCTGGCAACGGTCCGCGCGGCCTACACCCGCGCCGCGC 17340
        T  P  I  P  D  A  D  L  A  T  V  R  A  A  Y  T  R  A  A  L
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17341 TCGCGCTGCCGTGGCGAGAGGGCGACATCATGCTGGTCGACAACCTGAGGATGGCCCACG 17400
        A  L  P  W  R  E  G  D  I  M  L  V  D  N  L  R  M  A  H  G

17401 GCCGCGAGCCCTTCACCGGCGAGCGCCGCGTACTCGTCGCGATGACCTCGGCGGACTCAT 17460
        R  E  P  F  T  G  E  R  R  V  L  V  A  M  T  S  A  D  S  *

17461 GAGCCGTGCCGACGCATCGGCACGCCGTCCTCCCGTCGGGGCGCTACCATCGCCGCTGTC 17520

17521 TCGGCCATCACCCCACCCGGGCGGAGGCAACCGGCCGTGCACATCCCCGCCGTGGTCGCC 17580

17581 ACGGCACGCGCGATCACCCGCGCCATGACCGCCCAGCCCGTTGTCACATCTGCGGAGGCG 17640

17641 CCGCGATGACAGAGGTCCGAGGTGAACTGATCCGGGCGCTGCCGGGTGTGCTGGAGGCGC 17700
        M  T  E  V  R  G  E  L  I  R  A  L  P  G  V  L  E  A  R
        (orf40)

17701 GTGCGGCGCGGGCGGGGCACACGACCGCCTTCCTCGACGCACGACGGTGTGTCACGTACC 17760
        A  A  R  A  G  H  T  T  A  F  L  D  A  R  R  C  V  T  Y  R

17761 GGGAGTTGGAGGCGCGCACCCGCCGGCTGGCGGGGTCACCTGGTGCGGTTGGGGGTGCGC 17820
        E  L  E  A  R  T  R  R  L  A  G  S  P  G  A  V  G  G  A  Q

17821 AGGGGCAGACCGGGTGGCGCTCGTCAATGGGCAACCGGGGTGGAGATGGCGGAGGGTTCC 17880
        G  Q  T  G  W  R  S  S  M  G  N  R  G  G  D  G  G  G  P  P

17881 CTCCCCGGTGCTGCGGGCCGGAGCGGTAGGGGTGCCGCTCGATTCCGGGGCCACGGACGC 17940
        P  R  C  C  G  P  E  R  *  G  C  R  S  I  P  G  P  R  T  R

17941 GGAGCTCGCGTACTTCCTCGACGACTGTGGAGCGGTGGCGGTGGTCACCGAGGAGACGCT 18000
        S  S  R  T  S  S  T  T  V  E  R  W  R  W  S  P  R  R  R  C

18001 GCTGCCGCGGGTCTCGCGATCGGCGGGCGTACGGATCCTGGTGGGGGGTTCGGACGCCGT 18060
        C  R  G  S  R  D  R  R  A  Y  G  S  W  W  G  V  R  T  P  S

18061 CCCGGAGGGAGCGGCTGCCGGCATCCACTCCTTCGAGCGGCTCGCGGCGTCGGATCCGGG 18120
        R  R  E  R  L  P  A  S  T  P  S  S  G  S  R  R  R  I  R  G

18121 GTGCGCGCCACGGGACGACCTCGGCCTCGACGAGCCGGCCTGGATCCTCTACACGTCGGG 18180
        A  R  H  G  T  T  S  A  S  T  S  R  P  G  S  S  T  R  R  G

18181 GACCACGGGCCGGAGCAAGGGCGTGGTCTGCGGCCAGCGCGCCGCGCTGTGGTCCGTGGC 18240
        P  R  A  G  A  R  A  W  S  A  A  S  A  P  R  C  G  P  W  R

18241 GGCGGCGTACGTGCCGTCGTGGGGTCTGGGGCCGCAGGACCGGCTGTTGTGGCCGCTGCC 18300
        R  R  T  C  R  R  G  V  W  G  R  R  T  G  C  C  G  R  C  P

18301 CATGTTCCACGCCTACGCGCACTCGCTGTGCCTGCTCGGGGTGGTGGCCGTGGGCGCGAG 18360
        C  S  T  P  T  R  T  R  C  A  C  S  G  W  W  P  W  A  R  A

18361 CGCGTACCTCCTCGACCGGGGCGCGAGCGTCGTCCGGGCGCTTGAGGAACAGCGGTGCAG 18420
        R  T  S  S  T  G  A  R  A  S  S  G  R  L  R  N  S  G  A  A

18421 CGTCGTGGCCGGTGTACCCGCCACCTACCGCCTGCTCACGAGCGCCTTCCGCGACGCCCC 18480
        S  W  P  V  Y  P  P  P  T  A  C  S  R  A  P  S  A  T  P  P

18481 CCGGCCACCGGCCGGCCTGCGACTGTGCGTCACCGGGGGCTGCGCCGTGCCCGCCGGGGC 18540
        G  H  R  P  A  C  D  C  A  S  P  G  A  A  P  C  P  P  G  L

18541 TGCGGGCGGACGTTGAGGAGCTGCTGGGCGTCCCGCTGCTCGACGGTTACGGCAGTACCG 18600
        R  A  D  V  E  E  L  L  G  V  P  L  L  D  G  Y  G  S  T  E
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18601 AGACCTGCGGCAAGATCACGGTTGAGCGGCTCGGCGGCTCCCGGGAGGGCGGTTGCCGGG  18660
        T  C  G  K  I  T  V  E  R  L  G  G  S  R  E  G  G  C  R
```

SEQ ID NO: 3 BLM gene PPTase ORFS 41

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    1 GGATCCTGCGCTACCCGGACTTCGCCCAGTGGTGCGGCACCGAGCTCACCGCCGACTGGCACGTCCGCTTCCGGGCCGCC   80
   81 GCCGCGGTCTACGGGCATCTGCACATCCCCCGCGTGACCCGGTACGACGGCGTCCGCTTCGAGGAGGTGTCGGTCGGCTA  160
  161 CCCGCGCGAGTGGCGGCCCCGGCCGCCCCGCGAGCCGCTCCGGCAGATCCTGCCCCAGCCCGTCGACGAGCCGGGAGCCC  240
  241 TCTGGTGATCGCCGCCCTCCTGCCCTCCTGGGCCGTCACCGAACACGCCTTCACCGACGCCCCGGACGACCCGGTGAGCC  320
    1     M  I  A  A  L  L  P  S  W  A  V  T  E  H  A  F  T  D  A  P  D  D  P  V  S  L   26
  321 TCCTCTTCCCCGAGGAGGCCGCCCACGTCGCCCGCGCCGTCCCCAAGCGCCTGCACGAGTTCGCCACCGTCCGGGTGTGC  400
   27   L  F  P  E  E  A  A  H  V  A  R  A  V  P  K  R  L  H  E  F  A  T  V  R  V  C    52
  401 GCCCGCGCCGCCCTCGGCCGGCTGGGCCTCCCGCCCGGTCCGCTGCTGCCCGGCCGACGGGGCGCGCCGAGCTGGCCGGA  480
   53 A  R  A  A  L  G  R  L  G  L  P  P  G  P  L  L  P  G  R  R  G  A  P  S  W  P  D   79
  481 CGGGGTGGTGGGGAGCATGACGCACTGTCAGGGCTTCCGGGGCGCCGCGGTCGCCCGGGCCGCCGACGCCGCGTCGCTCG  560
   80  G  V  V  G  S  M  T  H  C  Q  G  F  R  G  A  A  V  A  R  A  A  D  A  A  S  L  G  106
  561 GGATAGACGCCGAGCCGAACGGGCCGCTCCCGGACGGCGTCCTCGCCATGGTCTCGCTGCCGTCCGAGCGCGAGTGGCTC  640
  107   I  D  A  E  P  N  G  P  L  P  D  G  V  L  A  M  V  S  L  P  S  E  R  E  W  L   132
  641 GCCGGACTGGCGGCCCGCCGGCCGGACGTGCACTGGGACCGGCTGCTGTTCAGCGCCAAGGAGAGCGTCTTCAAGGCGTG  720
  133 A  G  L  A  A  R  R  P  D  V  H  W  D  R  L  L  F  S  A  K  E  S  V  F  K  A  W   159
  721 GTACCCGCTGACCGGCCTGGAGCTGGACTTCGACGAGGCCGAGCTGGCCGTCGATCCGGACGCCGGGACGTTCACGGCCC  800
  160  Y  P  L  T  G  L  E  L  D  F  D  E  A  E  L  A  V  D  P  D  A  G  T  F  T  A  R  186
  801 GGCTGCTGGTGCCGGGACCGGTGGTCGGCGGCCGTCGGCTGGACGGGTTCGAGGGGCGCTGGGCGGCGGGCGAGGGCCTC  880
  187   L  L  V  P  G  P  V  V  G  G  R  R  L  D  G  F  E  G  R  W  A  A  G  E  G  L   212
  881 GTCGTCACGGCCATCGCCGTCGCGGCGCCGGCCGGTACCGCGGAGGAATCGGCGGAAGGGGCCGGGAAGGAAGCGACTGC  960
  213 V  V  T  A  I  A  V  A  A  P  A  G  T  A  E  E  S  A  E  G  A  G  K  E  A  T  A  239
  961 GGACGACCGGACCGCCGTCCCGTAAACCGCCCCGAACACCGGCGTGGCGCCCGCCGACCGTGTCGGGGGCGCCACGAACG 1040
  240  D  D  R  T  A  V  P  *                                                          247
 1041 GGCGCCGGCCCGGCGGGCCCTCCGCCGTGCGGAGCGGAGGCCCGGCGCGGACGCGCCCGGTGTCGTCGGATACGTGCGTC 1120
 1121 AGTCGGCGACGCAGACGTTGCCGTTGGTCGAGTTGAGCAGCCCGACGATGTCGATGGTGTTGCCGCAGAGGTTGATGGGG 1200
 1201 ATGTGGACGGGGATCTGGATGACGTTGCCCGAGACGACGCCCGGGGAGCCGACGGCCGCCCCCTTGGCGTTCGAGTCGGC 1280
 1281 GAGGGCGGTGCCGGAGACGCCGGCGAGCGCCGTGCCCACGGTGGCGGTGAGGGCCGCTGCCTTGGCGATTCGTGACATGG 1360
 1361 GGTGACACCTTCGTTCGGTCTGACAGGGTCGAGCTCACGGCCTCTGACGGCCGGGAGCCCGGATCAACGCCCGATCACCC 1440
 1441 CGAAGGTTTCGAATCGTGCGGCGGACGGGTGACCGGCGGCCGAACGGCCTCGCCGGGCCCCCGGAAGGTGCCATGACGTC 1520
 1521 CGTGCGCCATCTGTACAGCCCGGTCCCGCGCCGCGTACAAGGGACGGACGGACGGCCGGTGGACGGACGACCGGCGGGGA 1600
 1601 GGGGAGGCCATGAGCCGGATCGCGATCGTCGGGGCGGGTCAGGCCGGACTGCATCTGGCGCTGGGGCTGCTGGGGGCGGG 1680
 1681 GAGCGGCTCTTCCCGTCACGAGGTGCTGCTCGTGTCCGACGGGACGCCGGACGAGATCCGCGCCGGGCGGGTGCGGTCGA 1760
 1761 C  1761
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/00445

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12N 9/00
US CL : 435/183

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/183, 536/23.2, 435/320.1, 435/252.35

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SUGIYAMA et al. Characterisation by molecular cloning of two genes from Streptomyces verticillus encoding resistance to bleomycin. Gene 1994, Vol.151, pages 11-16.	1-45, 65-69, 71-73
A	CALCUTT et al. Gene organization in the bleomycin-resistance region of the producer organism Streptomyces verticillus. Gene. 1994, Vol.151, pages 17-21	1-45, 65-69 71-73

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
29 MARCH 2000

Date of mailing of the international search report
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Form PCT/ISA/210 (second sheet) (July 1998)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/00445

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-45, 65-69, and 71-73 to the extent they read on ORF8

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/00445

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

STN
search terms: bleomycin, gene, operon, orf, open reading frame, cluster, aureus, verticillus, host cell, polyketide synthase, PKS

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s)1-45, 65-69, and 71-73, drawn to isolated nucleic acids, gene clusters, multi-functional protein complexes, polypeptides, expression vectors, and host cells, to the extent that these products read on ORF 8.
Group II, claim(s)1-45, 65-69, and 71-73, drawn to isolated nucleic acids, gene clusters, multi-functional protein complexes, polypeptides, expression vectors, and host cells, to the extent that these products read on ORF 9.
Group III, claim(s)1-45, 65-69, and 71-73, drawn to isolated nucleic acids, gene clusters, multi-functional protein complexes, polypeptides, expression vectors, and host cells, to the extent that these products read on ORF 10.
Group IV, claim(s)1-45, 65-69, and 71-73, drawn to isolated nucleic acids, gene clusters, multi-functional protein complexes, polypeptides, expression vectors, and host cells, to the extent that these products read on ORF 11.
Group V, claim(s)1-45, 65-69, and 71-73, drawn to isolated nucleic acids, gene clusters, multi-functional protein complexes, polypeptides, expression vectors, and host cells, to the extent that these products read on ORF 12.
Group VI, claim(s)1-45, 65-69, and 71-73, drawn to isolated nucleic acids, gene clusters, multi-functional protein complexes, polypeptides, expression vectors, and host cells, to the extent that these products read on ORF 13.
Group VII, claim(s)1-45, 65-69, and 71-73, drawn to isolated nucleic acids, gene clusters, multi-functional protein complexes, polypeptides, expression vectors, and host cells, to the extent that these products read on ORF 14.
Group VIII, claim(s)1-45, 65-69, and 71-73, drawn to isolated nucleic acids, gene clusters, multi-functional protein complexes, polypeptides, expression vectors, and host cells, to the extent that these products read on ORF 15.
Group IX, claim(s)1-45, 65-69, and 71-73, drawn to isolated nucleic acids, gene clusters, multi-functional protein complexes, polypeptides, expression vectors, and host cells, to the extent that these products read on ORF 16.
Group X, claim(s)1-45, 65-69, and 71-73, drawn to isolated nucleic acids, gene clusters, multi-functional protein complexes, polypeptides, expression vectors, and host cells, to the extent that these products read on ORF 17.
Group XI, claim(s)1-45, 65-69, and 71-73, drawn to isolated nucleic acids, gene clusters, multi-functional protein complexes, polypeptides, expression vectors, and host cells, to the extent that these products read on ORF 18.
Group XII, claim(s)1-45, 65-69, and 71-73, drawn to isolated nucleic acids, gene clusters, multi-functional protein complexes, polypeptides, expression vectors, and host cells, to the extent that these products read on ORF 19.
Group XIII, claim(s)1-45, 65-69, and 71-73, drawn to isolated nucleic acids, gene clusters, multi-functional protein complexes, polypeptides, expression vectors, and host cells, to the extent that these products read on ORF 20.
Group XIV, claim(s)1-45, 65-69, and 71-73, drawn to isolated nucleic acids, gene clusters, multi-functional protein complexes, polypeptides, expression vectors, and host cells, to the extent that these products read on ORF 21.
Group XV, claim(s)1-45, 65-69, and 71-73, drawn to isolated nucleic acids, gene clusters, multi-functional protein complexes, polypeptides, expression vectors, and host cells, to the extent that these products read on ORF 22.
Group XVI, claim(s)1-45, 65-69, and 71-73, drawn to isolated nucleic acids, gene clusters, multi-functional protein complexes, polypeptides, expression vectors, and host cells, to the extent that these products read on ORF 23.
Group XVII, claim(s)1-45, 65-69, and 71-73, drawn to isolated nucleic acids, gene clusters, multi-functional protein complexes, polypeptides, expression vectors, and host cells, to the extent that these products read on ORF 24.
Group XVIII, claim(s)1-45, 65-69, and 71-73, drawn to isolated nucleic acids, gene clusters, multi-functional protein complexes, polypeptides, expression vectors, and host cells, to the extent that these products read on ORF 25.
Group XIX, claim(s)1-45, 65-69, and 71-73, drawn to isolated nucleic acids, gene clusters, multi-functional protein complexes, polypeptides, expression vectors, and host cells, to the extent that these products read on ORF 26.
Group XX, claim(s)1-45, 65-69, and 71-73, drawn to isolated nucleic acids, gene clusters, multi-functional protein complexes, polypeptides, expression vectors, and host cells, to the extent that these products read on ORF 27.
Group XXI, claim(s)1-45, 65-69, and 71-73, drawn to isolated nucleic acids, gene clusters, multi-functional protein complexes, polypeptides, expression vectors, and host cells, to the extent that these products read on ORF 28.
Group XXII, claim(s)1-45, 65-69, and 71-73, drawn to isolated nucleic acids, gene clusters, multi-functional protein complexes, polypeptides, expression vectors, and host cells, to the extent that these products read on ORF 29.
Group XXIII, claim(s)1-45, 65-69, and 71-73, drawn to isolated nucleic acids, gene clusters, multi-functional protein complexes, polypeptides, expression vectors, and host cells, to the extent that these products read on ORF 30.
Group XXIV, claim(s)1-45, 65-69, and 71-73, drawn to isolated nucleic acids, gene clusters, multi-functional protein

complexes, polypeptides, expression vectors, and host cells, to the extent that these products read on ORF 31.
Group XXV, claim(s)1-45, 65-69, and 71-73, drawn to isolated nucleic acids, gene clusters, multi-functional protein complexes, polypeptides, expression vectors, and host cells, to the extent that these products read on ORF 32.
Group XXVI, claim(s)1-45, 65-69, and 71-73, drawn to isolated nucleic acids, gene clusters, multi-functional protein complexes, polypeptides, expression vectors, and host cells, to the extent that these products read on ORF 33.
Group XXVII, claim(s)1-45, 65-69, and 71-73, drawn to isolated nucleic acids, gene clusters, multi-functional protein complexes, polypeptides, expression vectors, and host cells, to the extent that these products read on ORF 34.
Group XXVIII, claim(s)1-45, 65-69, and 71-73, drawn to isolated nucleic acids, gene clusters, multi-functional protein complexes, polypeptides, expression vectors, and host cells, to the extent that these products read on ORF 35.
Group XXIX, claim(s)1-45, 65-69, and 71-73, drawn to isolated nucleic acids, gene clusters, multi-functional protein complexes, polypeptides, expression vectors, and host cells, to the extent that these products read on ORF 36.
Group XXX, claim(s)1-45, 65-69, and 71-73, drawn to isolated nucleic acids, gene clusters, multi-functional protein complexes, polypeptides, expression vectors, and host cells, to the extent that these products read on ORF 37.
Group XXXI, claim(s)1-45, 65-69, and 71-73, drawn to isolated nucleic acids, gene clusters, multi-functional protein complexes, polypeptides, expression vectors, and host cells, to the extent that these products read on ORF 38.
Group XXXII, claim(s)1-45, 65-69, and 71-73, drawn to isolated nucleic acids, gene clusters, multi-functional protein complexes, polypeptides, expression vectors, and host cells, to the extent that these products read on ORF 39.
Group XXXIII, claim(s)1-45, 65-69, and 71-73, drawn to isolated nucleic acids, gene clusters, multi-functional protein complexes, polypeptides, expression vectors, and host cells, to the extent that these products read on ORF 40.
Group XXXIV, claim(s)1-45, 65-69, and 71-73, drawn to isolated nucleic acids, gene clusters, multi-functional protein complexes, polypeptides, expression vectors, and host cells, to the extent that these products read on ORF 41.

Group XXXV, claims 46-57, drawn to methods of chemically modifying a biological molecule using a polypeptide encoded by ORF 8.
Group XXXVI, claims 46-57, drawn to methods of chemically modifying a biological molecule using a polypeptide encoded by ORF 9.
Group XXXVII, claims 46-57, drawn to methods of chemically modifying a biological molecule using a polypeptide encoded by ORF 10.
Group XXXVIII, claims 46-57, drawn to methods of chemically modifying a biological molecule using a polypeptide encoded by ORF 11.
Group XXXIX, claims 46-57, drawn to methods of chemically modifying a biological molecule using a polypeptide encoded by ORF 12.
Group XL, claims 46-57, drawn to methods of chemically modifying a biological molecule using a polypeptide encoded by ORF 13.
Group XLI, claims 46-57, drawn to methods of chemically modifying a biological molecule using a polypeptide encoded by ORF 14.
Group XLII, claims 46-57, drawn to methods of chemically modifying a biological molecule using a polypeptide encoded by ORF 15.
Group XLIII, claims 46-57, drawn to methods of chemically modifying a biological molecule using a polypeptide encoded by ORF 16.
Group XLIV, claims 46-57, drawn to methods of chemically modifying a biological molecule using a polypeptide encoded by ORF 17.
Group XLV, claims 46-57, drawn to methods of chemically modifying a biological molecule using a polypeptide encoded by ORF 18.
Group XLVI, claims 46-57, drawn to methods of chemically modifying a biological molecule using a polypeptide encoded by ORF 19.
Group XLVII, claims 46-57, drawn to methods of chemically modifying a biological molecule using a polypeptide encoded by ORF 20.
Group XLVIII, claims 46-57, drawn to methods of chemically modifying a biological molecule using a polypeptide encoded by ORF 21.
Group XLIX, claims 46-57, drawn to methods of chemically modifying a biological molecule using a polypeptide encoded by ORF 22.
Group L, claims 46-57, drawn to methods of chemically modifying a biological molecule using a polypeptide encoded by ORF 23.
Group LI, claims 46-57, drawn to methods of chemically modifying a biological molecule using a polypeptide encoded by ORF 24.
Group LII, claims 46-57, drawn to methods of chemically modifying a biological molecule using a polypeptide encoded by ORF 25.
Group LIII, claims 46-57, drawn to methods of chemically modifying a biological molecule using a polypeptide encoded by ORF 26.

Group LIV, claims 46-57, drawn to methods of chemically modifying a biological molecule using a polypeptide encoded by ORF 27.
Group LV, claims 46-57, drawn to methods of chemically modifying a biological molecule using a polypeptide encoded by ORF 28.
Group LVI, claims 46-57, drawn to methods of chemically modifying a biological molecule using a polypeptide encoded by ORF 29.
Group LVII, claims 46-57, drawn to methods of chemically modifying a biological molecule using a polypeptide encoded by ORF 30.
Group LVIII, claims 46-57, drawn to methods of chemically modifying a biological molecule using a polypeptide encoded by ORF 31.
Group LIX, claims 46-57, drawn to methods of chemically modifying a biological molecule using a polypeptide encoded by ORF 32.
Group LX, claims 46-57, drawn to methods of chemically modifying a biological molecule using a polypeptide encoded by ORF 33.
Group LXI, claims 46-57, drawn to methods of chemically modifying a biological molecule using a polypeptide encoded by ORF 34.
Group LXII, claims 46-57, drawn to methods of chemically modifying a biological molecule using a polypeptide encoded by ORF 35.
Group LXIII, claims 46-57, drawn to methods of chemically modifying a biological molecule using a polypeptide encoded by ORF 36.
Group LXIV, claims 46-57, drawn to methods of chemically modifying a biological molecule using a polypeptide encoded by ORF 37.
Group LXV, claims 46-57, drawn to methods of chemically modifying a biological molecule using a polypeptide encoded by ORF 38.
Group LXVI, claims 46-57, drawn to methods of chemically modifying a biological molecule using a polypeptide encoded by ORF 39.
Group LXVII, claims 46-57, drawn to methods of chemically modifying a biological molecule using a polypeptide encoded by ORF 40.
Group LXVIII, claims 46-57, drawn to methods of chemically modifying a biological molecule using a polypeptide encoded by ORF 41.

Group LXIX, claims 58-61, drawn to methods of coupling a first amino acid to a second amino acid using a polypeptide encoded by ORF 16.
Group LXX, claims 58-61, drawn to methods of coupling a first amino acid to a second amino acid using a polypeptide encoded by ORF 17.
Group LXXI, claims 58-61, drawn to methods of coupling a first amino acid to a second amino acid using a polypeptide encoded by ORF 21.
Group LXXII, claims 58-61, drawn to methods of coupling a first amino acid to a second amino acid using a polypeptide encoded by ORF 22.
Group LXXIII, claims 58-61, drawn to methods of coupling a first amino acid to a second amino acid using a polypeptide encoded by ORF 23.
Group LXXIV, claims 58-61, drawn to methods of coupling a first amino acid to a second amino acid using a polypeptide encoded by ORF 25.
Group LXXV, claims 58-61, drawn to methods of coupling a first amino acid to a second amino acid using a polypeptide encoded by ORF 26.
Group LXXVI, claims 58-61, drawn to methods of coupling a first amino acid to a second amino acid using a polypeptide encoded by ORF 32.
Group LXXVII, claims 58-61, drawn to methods of coupling a first amino acid to a second amino acid using a polypeptide encoded by ORF 38.

Group LXXVIII, claims 62-63, drawn to methods of coupling a first fatty acid to a second fatty acid using a polypeptide encoded by ORF 8.
Group LXXIX, claims 62-63, drawn to methods of coupling a first fatty acid to a second fatty acid using a polypeptide encoded by ORF 9.
Group LXXX, claims 62-63, drawn to methods of coupling a first fatty acid to a second fatty acid using a polypeptide encoded by ORF 10.
Group LXXXI, claims 62-63, drawn to methods of coupling a first fatty acid to a second fatty acid using a polypeptide encoded by ORF 11.
Group LXXXII, claims 62-63, drawn to methods of coupling a first fatty acid to a second fatty acid using a polypeptide encoded by ORF 12.

Group LXXXIII, claims 62-63, drawn to methods of coupling a first fatty acid to a second fatty acid using a polypeptide encoded by ORF 13.
Group LXXXIV, claims 62-63, drawn to methods of coupling a first fatty acid to a second fatty acid using a polypeptide encoded by ORF 14.
Group LXXXV, claims 62-63, drawn to methods of coupling a first fatty acid to a second fatty acid using a polypeptide encoded by ORF 15.
Group LXXXVI, claims 62-63, drawn to methods of coupling a first fatty acid to a second fatty acid using a polypeptide encoded by ORF 16.
Group LXXXVII, claims 62-63, drawn to methods of coupling a first fatty acid to a second fatty acid using a polypeptide encoded by ORF 17.
Group LXXXVIII, claims 62-63, drawn to methods of coupling a first fatty acid to a second fatty acid using a polypeptide encoded by ORF 18.
Group LXXXIX, claims 62-63, drawn to methods of coupling a first fatty acid to a second fatty acid using a polypeptide encoded by ORF 19.
Group XC, claims 62-63, drawn to methods of coupling a first fatty acid to a second fatty acid using a polypeptide encoded by ORF 20.
Group XCI, claims 62-63, drawn to methods of coupling a first fatty acid to a second fatty acid using a polypeptide encoded by ORF 21.
Group XCII, claims 62-63, drawn to methods of coupling a first fatty acid to a second fatty acid using a polypeptide encoded by ORF 22.
Group XCIII, claims 62-63, drawn to methods of coupling a first fatty acid to a second fatty acid using a polypeptide encoded by ORF 23.
Group XCIV, claims 62-63, drawn to methods of coupling a first fatty acid to a second fatty acid using a polypeptide encoded by ORF 24.
Group XCV, claims 62-63, drawn to methods of coupling a first fatty acid to a second fatty acid using a polypeptide encoded by ORF 25.
Group XCVI, claims 62-63, drawn to methods of coupling a first fatty acid to a second fatty acid using a polypeptide encoded by ORF 26.
Group XCVII, claims 62-63, drawn to methods of coupling a first fatty acid to a second fatty acid using a polypeptide encoded by ORF 27.
Group XCVIII, claims 62-63, drawn to methods of coupling a first fatty acid to a second fatty acid using a polypeptide encoded by ORF 28.
Group XCIX, claims 62-63, drawn to methods of coupling a first fatty acid to a second fatty acid using a polypeptide encoded by ORF 29.
Group C, claims 62-63, drawn to methods of coupling a first fatty acid to a second fatty acid using a polypeptide encoded by ORF 30.
Group CI, claims 62-63, drawn to methods of coupling a first fatty acid to a second fatty acid using a polypeptide encoded by ORF 31.
Group CII, claims 62-63, drawn to methods of coupling a first fatty acid to a second fatty acid using a polypeptide encoded by ORF 32.
Group CIII, claims 62-63, drawn to methods of coupling a first fatty acid to a second fatty acid using a polypeptide encoded by ORF 33.
Group CIV, claims 62-63, drawn to methods of coupling a first fatty acid to a second fatty acid using a polypeptide encoded by ORF 34.
Group CV, claims 62-63, drawn to methods of coupling a first fatty acid to a second fatty acid using a polypeptide encoded by ORF 35.
Group CVI, claims 62-63, drawn to methods of coupling a first fatty acid to a second fatty acid using a polypeptide encoded by ORF 36.
Group CVII, claims 62-63, drawn to methods of coupling a first fatty acid to a second fatty acid using a polypeptide encoded by ORF 37.
Group CVIII, claims 62-63, drawn to methods of coupling a first fatty acid to a second fatty acid using a polypeptide encoded by ORF 38.
Group CIX, claims 62-63, drawn to methods of coupling a first fatty acid to a second fatty acid using a polypeptide encoded by ORF 39.
Group CX, claims 62-63, drawn to methods of coupling a first fatty acid to a second fatty acid using a polypeptide encoded by ORF 40.
Group CXI, claims 62-63, drawn to methods of coupling a first fatty acid to a second fatty acid using a polypeptide encoded by ORF 41.

Group CXII, claim 64, drawn to methods of producing a bleomycin or bleomycin analog using ORF 8.
Group CXIII, claim 64, drawn to methods of producing a bleomycin or bleomycin analog using ORF 9.

Group CXIV, claim 64, drawn to methods of producing a bleomycin or bleomycin analog using ORF 10.
Group CXV, claim 64, drawn to methods of producing a bleomycin or bleomycin analog using ORF 11.
Group CXVI, claim 64, drawn to methods of producing a bleomycin or bleomycin analog using ORF 12.
Group CXVII, claim 64, drawn to methods of producing a bleomycin or bleomycin analog using ORF 13.
Group CXVIII, claim 64, drawn to methods of producing a bleomycin or bleomycin analog using ORF 14.
Group CXIX, claim 64, drawn to methods of producing a bleomycin or bleomycin analog using ORF 15.
Group CXX, claim 64, drawn to methods of producing a bleomycin or bleomycin analog using ORF 16.
Group CXXI, claim 64, drawn to methods of producing a bleomycin or bleomycin analog using ORF 17.
Group CXXII, claim 64, drawn to methods of producing a bleomycin or bleomycin analog using ORF 18.
Group CXXIII, claim 64, drawn to methods of producing a bleomycin or bleomycin analog using ORF 19.
Group CXXIV, claim 64, drawn to methods of producing a bleomycin or bleomycin analog using ORF 20.
Group CXXV, claim 64, drawn to methods of producing a bleomycin or bleomycin analog using ORF 21.
Group CXXVI, claim 64, drawn to methods of producing a bleomycin or bleomycin analog using ORF 22.
Group CXXVII, claim 64, drawn to methods of producing a bleomycin or bleomycin analog using ORF 23.
Group CXXVIII, claim 64, drawn to methods of producing a bleomycin or bleomycin analog using ORF 24.
Group CXXIX, claim 64, drawn to methods of producing a bleomycin or bleomycin analog using ORF 25.
Group CXXX, claim 64, drawn to methods of producing a bleomycin or bleomycin analog using ORF 26.
Group CXXXI, claim 64, drawn to methods of producing a bleomycin or bleomycin analog using ORF 27.
Group CXXXII, claim 64, drawn to methods of producing a bleomycin or bleomycin analog using ORF 28.
Group CXXXIII, claim 64, drawn to methods of producing a bleomycin or bleomycin analog using ORF 29.
Group CXXXIV, claim 64, drawn to methods of producing a bleomycin or bleomycin analog using ORF 30.
Group CXXXV, claim 64, drawn to methods of producing a bleomycin or bleomycin analog using ORF 31.
Group CXXXVI, claim 64, drawn to methods of producing a bleomycin or bleomycin analog using ORF 32.
Group CXXXVII, claim 64, drawn to methods of producing a bleomycin or bleomycin analog using ORF 33.
Group CXXXVIII, claim 64, drawn to methods of producing a bleomycin or bleomycin analog using ORF 34.
Group CXXXIX, claim 64, drawn to methods of producing a bleomycin or bleomycin analog using ORF 35.
Group CXL, claim 64, drawn to methods of producing a bleomycin or bleomycin analog using ORF 36.
Group CXLI, claim 64, drawn to methods of producing a bleomycin or bleomycin analog using ORF 37.
Group CXLII, claim 64, drawn to methods of producing a bleomycin or bleomycin analog using ORF 38.
Group CXLIII, claim 64, drawn to methods of producing a bleomycin or bleomycin analog using ORF 39.
Group CXLIV, claim 64, drawn to methods of producing a bleomycin or bleomycin analog using ORF 40.
Group CXLV, claim 64, drawn to methods of producing a bleomycin or bleomycin analog using ORF 41.

Group CXLVI, claim 70, drawn to methods of converting an apo-carrier protein to a holo-carrier protein using a phosphopantetheinyl transferase encoded by ORF 41.

The inventions listed as Groups I-CXLVI do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features. The MPEP states in Annex B (page AI-36) that

> "Unity of invention exists only when there is a special technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features. The expression 'special technical features' is defined in Rule 13.2 as meaning those technical features that define a contribution which each of the inventions, considered as a whole, makes over the prior art. The determination is made on the contents of the claims as interpreted in light of the description and drawings (if any)."

The following is the organization of the Groups:

Supergroup A (Groups I-XXXIV): each isolated nucleic acid comprising any one of ORFs 8 through 41 (34 separate groups);
Supergroup B (Groups XXXV-LXVIII): methods of chemically modifying a biological molecule using any one of ORFs 8 through 41 (34 separate groups);
Supergroup C (Groups LXIX-LXXVII): methods of coupling a first amino acid to a second amino acid using any one of ORFs 16, 17, 21-23, 25, 26, 32, or 38 (ORFs disclosed as encoding NRPSs)(9 separate groups);
Supergroup D (Groups LXXVIII-CXI): methods of coupling a first fatty acid to a second fatty acid using any one of

ORFs 8 through 41 (34 separate groups);
Supergroup E (Groups CXII-CXLV): methods of producing a bleomycin or bleomycin analog using any one of ORFs 8 through 41 (34 separate groups); and
Supergroup F (Group CXLVI): methods of converting an apo-carrier protein to a holo-carrier protein using ORF 41 (SEQ ID NO:3) (1 group).

1. The Groups within **Supergroup A** (Groups I-XXXIV) lack unity of invention for the following reasons:

The technical feature in Claim 1 is denoted by applicants' claim language, namely, "any one of *Blm* open reading frames (ORFs) 8 through 41" (emphasis added), indicating that each, individual ORF is an invention since any ONE open reading frame satisfies the claim. However, this technical feature is not a "special technical feature" within the meaning of PCT Rule 13.2 because it fails to distinguish over the prior art for the reasons set forth below.

At least 2 inventions in claim 1, and possibly more, do not contribute over the prior art upon a cursory, prior art search for the purposes of defining the unity of inventions. Sugiyama et al. (*Gene* 151 (1994) 11-16) and Calcutt et al. (*Gene* 151 (1994) 17-21) teach a 14.4 kb plasmid, pMSA-1 (see Sugiymama et al., page 13, Fig. 2), which contains the bleomycin resistance region from *Streptomyces verticillus* (ORFs 1-7); Calcutt et al. further suggest that "resistance and production functions may be clustered" (see page 21). Applicants identify ORF 41 as being located between ORF 1 and ORF 3 (see instant specification Fig. 13), and thus Sugiyama et al. inherently teaches the equivalent of ORF 41 in their pMSA-1. Additionally, applicants' own specification positions *blm*I (ORF 10) approximately 4 kb upstream of *blm*A as defined by Sugiyama et al. and Calcutt et al. (see instant specification, page 45, line 21). Clearly in applicants' Fig. 2, *blm*C (1.5 kb) and ORF 8 (1.28 kb) are downstream (closer to *blm*A) of *blm*I indicating that these ORFs are within 1.22 kb of *blm*A, and at least 3.8 kb upstream of *blm*A is taught in the pMSA-1 plasmid which would encompass all of ORF 8 and most of *blm*C.

Each technical feature in claim 1, i.e. ORFs 8 through 41, encodes a unique polypeptide with a unique function, with respect to the other ORFs, as supported by applicants' specification in Tables I and II which notes the distinct enzymatic activities of the disclosed ORFs. Thus, ORFs 8 through 41 lack not only the same, but also a corresponding special technical feature.

The 34 inventions in Claim 1, as defined by the 34 ORFs, are 34 isolated nucleic acids which, when considered as a whole, do not contribute a common special technical feature over the prior art. These 34 inventions are merely products, namely nucleic acids, which share the basic chemical construction of nucleic acids (deoxyribose sugar and phosphate backbone with one of five nucleoside bases attached). While these inventions may share utility, when coupled all together, for the production of bleomycin, that utility cannot be considered a special technical feature since it is neither expressly claimed nor clearly identified in Claim 1.

2. The Groups within **Supergroup B** (Groups XXXV-LXVIII) lack unity of invention for the following reasons:

The methods of Supergroup B lack unity because said methods use wholly different reagents (different polypeptides encoding by different ORFs) to produce wholly different products (bleomycin analogs). In particular, the use of any one ORF in the methods of Supergroup B renders a distinct product because polypeptides encoded by each of ORFs 8 through 41, as represented in Groups XXXV-LXVIII, have different and distinct functions (see instant specification Table I and II).

3. The Groups within **Supergroup C** (Groups LXIX-LXXVII) lack unity of invention for reasons analgous to those stated in section 2 above pertaining to Supergroup B.

4. The Groups within **Supergroup D** (Groups LXXVIII-CXI) lack unity of invention for reasons analgous to those stated in section 2 above pertaining to Supergroup B.

5. The Groups within **Supergroup E** (Groups CXII-CXLV) lack unity of invention for reasons analgous to those stated in section 2 above pertaining to Supergroup B.

6. **Supergroup F** contains only one group, Group CXLVI; however, it is named a Supergroup for consistency. No lack of unity is found within Supergroup F.

7. Each member of Supergroup A lacks unity of invention with each member of Supergroups B-F for the following reasons:

The technical feature of each ORF in claim 1 is a nucleic acid which can encode a polypeptide used in one of Groups XXXV-CXLVI. However, the methods can be practiced in the absence of the isolated nucleic acids, and in fact, are practiced in bleomycin-producing bacteria. Thus, the nucleic acids and the methods of using the encoded proteins lack unity of invention.

8. Each member of Supergroups B-F lacks unity of invention with every other member because each Group within Supergroups B-F produce a wholly different and distinct product. These products can be bleomycin analogs, dipeptides, diketides, 2-component fatty acids, and numerous permutations of tri, tetra, etc. of said products. Thus, all these method groups lack unity of invention with each other.